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TP1: ANALYTICAL METHODS/MASS SPECTROMETRY

TP1.01

PREGNANCY ASSOCIATED PLASMA PROTEIN-A (PAPP-A) AND βHCG MEASUREMENT ON THE DELFIA XPRESS

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Interest in screening for Down syndrome in the first trimester of pregnancy with pregnancy associated plasma protein A (PAPP-A) and the beta subunit of hCG (βhCG) has recently increased. To cope with the associated rise in assay requests, automation of the assays was considered. To this end the performance of the Delfia Xpress tests for PAPP-A and βhCG was compared to that of the manual Delfia tests and median values were established.

Intra-assay variation for βhCG as determined from differences in duplicates ($n=104$) varied from 2.2% at concentrations below 26 μg/L to 1.0% in the range of 125–200 μg/L. Interassay CV's as determined from in-house pool sera were 3.7; 4.4 and 5.4% at 8.3; 36.3 and 136 μg/L, respectively ($n=70$). Linearity was good, carry-over was 0.0013% at 8000 μg/L. Agreement with the manual Delfia βhCG assay was excellent: βhCG[Xpress]$=0.97\times\beta$hCG[manual] -0.3 (Passing Bablok; $r=0.995$; $n=286$).

For PAPP-A interassay variation was 1.2% below 700 mU/L to 1.4% in the range of 6000–12,000 mU/L. Interassay CV's were 4.5; 3.7 and 3.6% at 90; 211 and 3990 mU/L, respectively. Linearity in the concentration range of 400–9000 mU/L was good. Agreement with the manual Delfia was excellent: PAPP-A[Xpress]$=$ $0.93\times$PAPP-A[manual] -19.9 ($2=0.998$; $n=286$).

For both assays, specimens could be drawn in serum tubes with or without separator gel. The relationship between median values and pregnancy duration has been established. As expected, the new medians correlated well with those previously determined with the manual assay.

We conclude that the performance of the Xpress assays for βHCG and PAPP-A warrants their introduction in clinical practice.

TP1.02

DIFFERENT ALBUMIN, DIFFERENT ASSOCIATIONS WITH ATHEROSCLEROTIC VASCULAR DISEASE?

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Microalbuminuria has been identified as an independent cardiovascular risk factor. Immunochemical urinary albumin assays only detect immunoreactive albumin whereas HPLC detects both immunoreactive and immuno-unreactive albumin. We compare these methods both analytically and in their relation with peripheral vascular disease (PVD).

We used urine samples that were collected for the PREVEND study. PVD was defined by an ankle-brachial index <0.9.

With nephelometry as reference method, we classified 998 subjects normoalbuminuric (NA, UAC <20 mg/L), 280 microalbuminuric (MA, UAC 20–200 mg/L), and 34 macroalbuminuric (MACRO, UAC>200 mg/L). HPLC measured more albumin compared to nephelometry in the lower albuminuria ranges: in NA 17.6 ± 10.3 vs. 6.8 ± 4.3 mg/L ($p<0.001$) for nephelometry; in MA 74.0 ± 51.8 vs. 58.9 ± 40.6 mg/L ($p<0.001$); in MACRO 399 ± 329 vs. 436 ± 371 mg/L ($p=0.048$). The prevalence of MA when assessed by HPLC was significantly higher than when assessed with nephelometry (42 vs. 21%, $p<0.05$), whereas the prevalence of PVD was comparable in both MA groups (9.6 vs. 9.3%, $p=$NS). Logistic regression analysis with PVD as outcome parameter revealed odds ratios (adjusted for age, gender, and other risk factors) of 2.0 (95%CI 1.2–3.4, $p<0.05$) and 3.3 (95%CI 1.1–9.8, $p<0.05$) respectively for MA and MACRO with HPLC, compared to 1.2 (95%CI, 0.7–2.1, $p=$NS) and 3.0 (95%CI, 1.1–8.2, $p<0.05$) with nephelometry. ROC-analysis showed similar sensitivity and specificity for both methods.

HPLC reveals higher urinary albumin concentrations than nephelometry, especially in the lower range, resulting in a higher prevalence of MA. Despite this, the relation with PVD remained similar for both methods. HPLC therefore identifies more subjects with a similar risk. This suggests that measurement of urinary albumin by HPLC can improve its predictive value for cardiovascular disease.

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TP1.03

THE ROUTINE AUTOMATED SOLID-PHASE EXTRACTION OF URINARY FREE CATECHOLAMINES USING THE GILSON ASPEC XL4

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The Gilson ASPEC XL4 is an automated solid-phase extraction (SPE) sample processor. Our aim was to automate our routine in-house manual extraction method for urinary-free catecholamines.
The manual assay required 1.0 mL sample mixed with HMBA and adjusted to pH 6.5. It was then transferred into a 0.5 cm i.d. Econo™ column containing 4 cm of Amberlite CG-50 resin. After acidifying with sulphuric acid, the free catecholamines were eluted with ammonium sulphate onto 50 mg alumina. The alumina was buffered to pH 8.6 and the adsorbed catecholamines recovered into phosphoric acid and analysed by HPLC-ECD.
We investigated the amounts of resin and alumina required for ASPEC automation and measured recovery, accuracy, linearity, %CVs and the speed of liquid dispensing.
Using in-house filled 3.0 mL SPE columns it was found that the optimal resin amount was 2.4 cm column in height. Neither a 1.0 mL column with the same amount of resin nor a 3.0 mL column with double the amount significantly improved recovery. The optimal amount of alumina was 50 mg contained in a 1.0 mL column.
Accuracy and linearity were assessed by Deming regression of NEQAS urines measured by ASPEC against stated NEQAS target values ($n=27$). Noradrenaline: ASPEC=0.970 NEQAS target −22; range 0–3000 nmol/L. Adrenaline: ASPEC=1.071 NEQAS target −0.8; range 0–1500 nmol/L. Dopamine: ASPEC=1.086 NEQAS target −168, range; 0–18,000 nmol/L.
The interbatch imprecision for Biorad Lyphochek™ Low ($n=16$) was noradrenaline 4.9%, adrenaline 6.5% and dopamine 8.4%.
The speed of liquid dispensing through the columns was optimised so that a batch of 3 standards, 3 QCs and 30 patients could be extracted into HPLC autosampler vials in 3.25 h.

TP1.04

EVALUATION OF THE PENTRA 400® CLINICAL CHEMISTRY ANALYSER ACCORDING TO THE NCCLS GUIDELINES: REFERENCE INSTRUMENT FOR QUALITY ASSURANCE IN ENZYME ACTIVITY MEASUREMENTS

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The analytical performance of the ABX Pentra 400® was evaluated for the determination of the catalytic activity of the enzymes ALP, ALT, AMYL, AST, CK, GGT and LD. The instrument was compared with Cobas® Fara II which serves as a reference instrument for enzyme measurements in a regional harmonisation program in combination with Roche reagents using fresh patient plasmas. Our goal was to implement a new analyser to replace the Cobas® Fara II system with comparable specifications. The goal of our harmonisation program is to ensure, that patient sera measured on routine analyser systems are equivalent to those obtained with either a reference method or a method calibrated by a certified reference material.
The precision of the ABX Pentra 400® was evaluated according to the EP5 guideline of the National Committee for Clinical Laboratory Standards (NCCLS). The enzyme methods according to the NCCLS were programmed using the option of the open channels. The accuracy of the Pentra 400® was determined according to the NCCLS EP9 guideline. Enzyme determinations were measured on the ABX Pentra 400® in combination with ABX reagents and also in combination with Roche reagents according to the IFCC methods. All CV values were within expected values. The correlation studies showed an excellent correlation between the two instruments and between both reagent lines.
We conclude that in our hands the ABX Pentra 400® instrument is an excellent instrument for enzyme measurements according to the IFCC and that this combination can be used as a reference system in proficiency testing of enzyme measurements.

TP1.05

THE INFLUENCE OF URINARY PIGMENTS ON THE DETERMINATION OF *N*-ACETYL-BETA-D-GLUCOSAMINIDASE

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The pigments and alkaline pH values of the urine make the determination of *N*-acetyl-beta-D-glucosaminidase (NAG, EC 3.2.1.30) activity unreliable. So, the influence of pigments on the determination of NAG activity and the procedures for its elimination were investigated.
NAG activity in human and rabbit urines with 2-methoxy-4-(2′-nitrovinyl)-phenyl-*N*-acetyl-beta-D-glucosoaminide as substrate was spectrophotometrically determined.
It was found that alkaline urine pH values influenced NAG activity in two ways.

1) NAG activity decreased due to enzyme instability with pH increase.
2) NAG activity increased because of the contributions of urinary pigments to the absorbance of 2-methoxy-4-(2′-nitrovinyl)-phenol (MNP) at 505 nm. It was shown that, beside the maximum in the range 350 to 360 nm of the absorption spectra of alkaline urines, there was a maximum in the range 380 to 460 nm. With increase of pH this maximum was shifted toward higher wavelengths and contributed to MNP absorption. On the other hand the maximum of MNP absorption was shifted toward lower wavelengths with pH increase.

In human urines the correction of the value of NAG activity for the influence of pigments (applying either of the applied procedures) should be performed when determining the NAG activity in weakly

acidic urines of intensive color (yellow-brown, orange). In alkaline urines: (a) pH 7 to 8, the increase in NAG activity due to the new absorption maximum of pigments and the decrease due to the alkaline pH was often evened out; (b) pH>8, often with yellow-brown and brown color, the determination (without or with correction for pigment influence) was unreliable.

TP1.06

FOCUS ON ELECTROLYTES AND LIPID PANEL PERFORMANCE – COMPARISON BETWEEN THE ABX PENTRA 400® AND THE COBAS® IINTEGRA 800

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The study aimed at validating the ABX Pentra 400 Clinical Chemistry system in correlation with Cobas® Integra 800. We particularly focused on electrolytes measured by potentiometry and lipid parameters performed in colorimetry.

The ABX Pentra 400 was compared to Integra 800 on the routine parameters: ions, enzymes, substrates. Fifty samples per parameter were randomly taken from the routine workload to evaluate Precision, Stability and Accuracy. We used a selection of lipaemic and icteric samples to assess the lipid panels.

R^2 was greater than 0.97 for the majority of parameters. On-board reagents and electrodes stability were in accordance with manufacturer's specifications. ABX Pentra 400 within-run and run-to-run precision is excellent for all parameters, especially for lipid and electrolyte panels.

For lipid panel, ABX Pentra 400 direct LDL was correlated with Cobas Integra 800 LDL methods: direct and calculated with the equation of Friedwald. Identified discrepancy was observed on some icteric and lipaemic samples. Correlation on sodium, potassium and chloride is excellent despite the differences in methods: direct potentiometry on ABX Pentra 400 and indirect potentiometry on Cobas Integra 800.

The ABX Pentra 400 is a very satisfactory routine analyser with user-friendly software and an emergency management panel that could be perfectible. It is a very flexible system and meets the laboratory requirements.

TP1.07

EVALUATION OF ADVIA CENTAUR SPECIFIC IgE ASSAY: KEY PERFORMANCE PARAMETERS FOR THE QUANTITATIVE DETERMINATION OF *PHLEUM PRATENSE* SPECIFIC IgE ANTIBODIES

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Accurate quantitative measurement of allergen-specific IgE for use in the diagnosis of allergic patients and assessment of outcome of allergy vaccination requires an assay with high sensitivity and specificity, plus the ability to quantify the wide range of biological responses over a wide dynamic range. Large clinical trials in seasonal allergic rhinoconjunctivitis using sublingual and subcutaneous immunotherapy have been initiated by ALK-Abelló and specific IgE is one of the important paraclinical parameters used, when assessing the outcome of immunotherapy.

This study evaluated the key assay performance characteristics: accuracy, precision, limit of detection, linearity, lot-to-lot consistency, calibration stability, reagent stability, sample integrity and finally interference of specific IgG and non-specific IgE antibodies.

The imprecision of the assay was determined using 5 controls at different concentrations of sIgE on 4 allergens analysed in replicates of 3 in 32 runs over a period of 5 weeks using 4 instruments. The CVpwr was 2.0 to 3.7% and CVtotal was 2.9 to 5.5%. Inaccuracy was −3.7% to 7.4%. Using NSB+3s the limit of detection was determined to 0.12 kU/L. The assay demonstrates interdilutional CVs<5% over the range with mean recovery of 105%. No interference of non-specific IgE up to 10.000 kU/L was seen and no interference was observed with specific IgG even up to 40 mg/mL. Control mean values for 13 consecutive lots of g6 allergen reagent were 98.9% with a total CV of 3.9%. Stored calibration was stable longer than the 4 weeks claimed.

Results clearly demonstrate that the ADVIA Centaur specific IgE assay system is well suited even for use in drug development and clinical trials with high standard demands.

TP1.08

SPECIFICITY OF METHODS FOR THE DETECTION OF MACROPROLACTIN

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Elevated levels of macroprolactin represents a relatively common cause of misdiagnosis and mismanagement of hyperprolactinaemic patients. The aim of this study was to assess the specificity of methods for removing high molecular mass prolactin complexes such as macroprolactin from serum prior to immunoassay.

Sera from 35 female patients with macroprolactinaemia were subjected to gel filtration chromatography (GFC) with quantitation of monomeric prolactin levels by DELFIA immunoassay. Residual serum prolactin levels were also measured following removal of prolactin complexes by pre-treatment of sera with polyethylene glycol (PEG), by ultrafiltration and by adsorption with protein G or protein A-Sepharose or anti-human IgG-agarose.

Total prolactin in the 35 sera examined ranged from 750–5747 mU/L with monomeric levels ranging from 126–440 mU/L. Of the five methods examined PEG correlated best with GFC ($r=0.76$) followed by protein G ($r=0.75$), protein A ($r=0.71$), anti-human IgG ($r=0.61$) and ultrafiltration ($r=0.43$). However, PEG treatment resulted in significantly lower residual prolactin levels (mean 72%) compared to GFC due to co-precipitation of some monomeric PRL by PEG. In contrast, sera pre-treated with protein G, protein A or anti-human IgG or sera subjected to ultrafiltration exhibited mean residual prolactin levels of 143%, 162%, 180% and 107%, respectively, relative to monomer levels obtained with GFC.

PEG and protein G pre-treatment yielded results that were closest to the GFC reference method. In instances where the use of PEG is not possible, pre-treatment with protein G appears to be a useful though

slightly more expensive alternative. However, protein G will not deplete serum of the rare forms of macroprolactin which do not contain IgG.

TP1.09

MEASUREMENT OF THROMBUS FORMATION: COMPARISON OF TWO METHODS

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There is a need for a proper test system of platelet adhesion for routine diagnostic testing and drug development. A suitable evaluation system should be able to test interactions of adhesive proteins, receptors and the subendothelial matrix, and its design should rely on the laws of rheology and the physiology of laminar flow. Parallel plate and rotational devices are increasingly used in research. We have evaluated qualitative and quantitative differences of platelet–collagen interaction under shear conditions to assess the conformity of results in the two chambers.

Heparinized blood samples were allowed to flow through human collagen type III surfaces in both parallel plate flow chamber (PPC) and cone and plate chamber (CPC) at a shear rate of 1000 s-1 for 2.5 min at 37 °C. Platelet deposition was characterized by surface coverage, average area, and height of thrombus. VWF distribution within thrombi was analysed with confocal laser scanning microscopy.

A significant increase in single platelet disappearance along with reduced surface coverage and average thrombus size observed in CPC hints at a higher rate of aggregation in this device, as opposed to PPC, where adhesion is the predominant process. Consequently, surface-specific platelet adhesion and aggregation are best evaluated with PPC, while comprehensive evaluation of surface-specific platelet adhesion and aggregation including aggregation in the flowing blood are best assessed using CPC. Therefore, we suggest that the comparison of adhesion devices is very much necessary for better understanding their diagnostic power, and the choice of chamber type as a diagnostic tool is purpose dependent.

TP1.10

PERFORMANCE OF THE IRIS iQ200 AUTOMATED URINE PARTICLE ANALYSER

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We assessed the performance of the Iris iQ200 Automated Urine Particle Analyser against advanced reference visual microscopy of 167 un-centrifuged specimens counted in chamber under phase contrast optics, and routine bright-field microscopy.

The iQ200 counted urinary red blood cells (RBC) at $r=0.894$ with Automated Particle Recognition (APR) alone and at $r=0.948$ after re-classification by human user. Sensitivity (Se) and specificity (Sp) figures against visual microscopy depend on the chosen cut-off limit: at 10 RBC × e6/L, Se was 73% with a Sp of 88%; at 20 RBC × e6/L, Se was 86% with a Sp of 96%, due to Poisson statistics. The corresponding performance for white blood cells (WBC) was $r=0.885$ with APR alone and $r=0.978$ after user re-classification. The correlations of counting after user re-classification were $r=0.927$ for squamous epithelial cells, $r=0.856$ for casts, and $r=0.706$ for non-squamous epithelial cells. The final detection of bacteria succeeded at a sensitivity of 64% with a specificity of 97% against the ordinal-scale reporting of visual microscopy. Between-day repeatability was 7% at 761 particles × e6/L and 34% at 21 particles × e6/L using fixed RBC suspensions, representing 2.2 to 2.7-fold dispersion of results as compared to the theoretical Poisson distribution.

Counting accuracy equalled or exceeded that of routine visual microscopy or flow cytometric technology. Captured images allow human re-classification and consultant reviews of observed findings.

TP1.11

A RAPID SAMPLE PRE-TREATMENT METHOD FOR REAL-TIME PCR DETECTION OF MICROORGANISMS

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Commonly used nucleic acid based methodologies for detection of specific organisms are technically compromised by the laborious and time-consuming steps of sample DNA extraction. In order to reduce the time needed for this step we have developed a rapid sample pre-treatment method based on filtration that is easily amenable to automation.

In the method, bacterial cells are purified from contaminating particles and/or PCR inhibitors by filtration, after which the purified cells are eluted directly into an amplification vessel without DNA extraction by a simple reversal of the direction of flow through the filter.

We have tested the filtration based technique with a variety of model analytes, e.g. *Listeria monocytogenes*, *Salmonella typhimurium* and *Bacillus subtilis*. Together with pre-dried assay reagents and a rapid PCR system, the approach allows detection of diverse microbial analytes in complex sample matrices in less than 30 min.

TP1.12

EVALUATION OF A D-DIMER ASSAY ON INNOTRAC Aio!™ IMMUNOANALYSER

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The aim of this study was to evaluate a D-Dimer kit designed for the Innotrac Aio!™ immunoanalyser. Innotrac Aio! is a complete immunoassay system consisting of an automated continuous access immunoanalyser and dry chemistry-based immunofluorometric assays with time-resolved fluorescence detection. In the D-Dimer

assay a sandwiching pair of monoclonal antibodies is used. Assay time is 18 min.
Detection limit was 0.02 mg/l. Within-run CV's at low, medium and high levels were all 4.6%. Between-day CV's at nine levels (0.1–39 mg/l) ranged from 5.3% to 10%. Dilution tests of three samples gave recoveries of 83% -113%. Correlation of Aio! D-Dimer to Auto · Dimer ® (Biopool) and to Tina-quant® D-Dimer (Roche) assays was as follows: Aio! D-Dimer=3.6 Auto · Dimer – 0.77, $r=0.95$, $n=140$ and Aio! D-Dimer=1.39 Tina-quant D-Dimer – 0.17, $r=0.94$, $n=84$.
Ten of the analysed samples were from patients with diagnosed deep vein thrombosis (DVT), 6 with pulmonary embolism (PE), 2 with disseminated intravascular coagulation (DIC) and 2 with DVT and PE. The D-Dimer values in DVT ranged from 0.91 mg/l to 48 mg/l, in PE from 0.48 mg/l to 81 mg/l, in DIC the results were 9.1 mg/l and 109 mg/l, and in DVT+PE 5.0 mg/l and 9.1 mg/l. The reference values according to the manufacturer are below 0.90 mg/l (<50 yrs). From the above patient values only one in the PE group was <0.90 mg/l. This sample gave a low value in all three methods. We conclude that the performance characteristics of the Innotrac Aio! D-Dimer assay are good and the method is well suited to routine use.

TP1.13

PERFORMANCE EVALUATION OF A HOMOGENOUS CARBOHYDRATE-DEFICIENT TRANSFERRIN IMMUNOASSAY FOR USE ON BN™ II AND BN PROSPEC® SYSTEMS

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This multicenter study evaluated the performance of a homogenous carbohydrate-deficient transferrin (CDT) immunoassay (N Latex CDT (in development) Dade Behring) for use on the BN™ Systems (Dade Behring).
N Latex CDT is a homogenous latex particle-enhanced agglutination inhibition assay requiring no sample pre-treatment. On the BN™ Systems the %CDT value is immediately available, if total transferrin is simultaneously performed. The evaluation included method comparisons versus the Axis-Shield %CDT method, precision studies in accordance with NCCLS EP5, determination of cut-off, diagnostic sensitivity and specificity with samples from both abstinent individuals and alcohol abusers plus interference studies (hyperlipaemia, transferrin variants and non-alcoholic liver diseases). HPLC, capillary electrophoresis or isoelectric focusing methods for CDT were used to resolve discrepant results. Field studies were performed in Aachen-Germany, Amersfoort-The Netherlands, Delft-The Netherlands, Gent-Belgium, Heidelberg-Germany, LaRiche-France, Stockholm-Sweden and Tours-France.
N Latex CDT showed superior performance compared to the Axis-Shield %CDT method with respect to specificity at comparable sensitivity. Genetic transferrin variant samples were classified correctly. Total imprecision was acceptable at all sites. A proficiency panel of 29 samples including both positive and negative samples was tested at seven sites using two lots of reagents on both analyzer platforms and yielded sample recoveries of 94 to 116%. Reference range studies in three European countries included more than 500 individuals of different age and gender groups.
N Latex CDT* showed superior performance when compared to Axis-Shield %CDT for the determination of %CDT. Fully automated processing on the BN™; Systems reduces hands-on time without the need of batch analyses.

TP1.14

XANTHOCHROMIA: IS THE ZERO LIMIT FOR NET OXYHAEMOGLOBIN ABSORBANCE TOO LOW?

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In 2003 national guidelines for analysis of cerebrospinal fluid (CSF) for bilirubin in suspected subarachnoid haemorrhage (SAH) were published. For samples with net bilirubin absorbance (NBA) of <0.007 AU, three categories were proposed dependent on net oxyhaemoglobin absorbance (NOA). These were NOA=0, NOA >0 but <0.1, and NOA=0.1 AU. A large proportion of our results falls into the second category.
Computer records covering 7 months in 2004 were examined. All (70) xanthochromia results were analysed and categorised according to the published guidelines. Information regarding the patients' care following receipt of the xanthochromia result was collected from case notes of patients that fell into category 2 (NBA<0.007, NOA>0 but <0.1) with a low NOA≤0.03.
The xanthochromia results revealed that 43% of samples (29 patients) with NBA of <0.007 AU fell into category 2, a 'grey area'. The majority had NOA levels much lower than 0.1 AU (27≤0.05, 22≤0.03, 18≤0.02). Of the 22 patients with NOA≤0.03, the case notes studied indicate that MRAngiography was performed on a minority and found to be normal, but that over 90% were discharged on simple analgesia with no further investigations performed.
The xanthochromia guidelines published in 2003 have resulted in a large number of patients being categorised in a grey area. The introduction of a NOA lower limit above zero would enable many of the patients in category 2 to be re-classified in category 1. Our data suggest that a larger study should be conducted to establish whether a NOA lower limit above zero could be introduced safely.

TP1.15

IS IT CORRECT TO LEAVE OUT THE FLUORIMETRIC METHODS FOR GLUTATHIONE ASSAY?

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Glutathione is the main intracellular antioxidant, which exists in two forms, the reduced and the oxidised. Changes in glutathione concentration serve as an important marker of the redox state inside

the cells. Various methods can be used to measure glutathione concentration in biological material, namely enzymatic, fluorimetric or HPLC. Nevertheless, due to the high sensitivity and specificity HPLC methods are the preferred method to analyse glutathione concentration, in spite of the cost and time demand.

The aim of our study was to establish analytical parameters of the spectrofluorimetric method, which should be suitable for the analysis of large numbers of samples. This method is based on the well-known principle—reaction between glutathione and o-phthaldialdehyde with subsequent production of strongly fluorescent isoindole-like product. To confirm selectivity of this method, we have analysed glutathione concentration in hepatocyte lysates using an HPLC method with spectrofluorimetric detection. Hepatocytes are appropriate cells to confirm specificity because of high intracellular level of thiols, which may interfere with the measurement of glutathione.

We have proved the specificity of the spectrofluorimetric method and found following analytical parameters: very good sensitivity (detection limit $c=2.5$ μM; linearity 5–250 μM), large accuracy (calibration curve $R^2=0.999$) and reproducibility (intra-assay CV%=2.5%, $n=10$). Our results show that spectrofluorimetric method is suitable for the analysis of glutathione levels.

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TP1.16

A COMPARISON BETWEEN THE BECKMAN COULTER ACCESS® OSTASE® (BONE-SPECIFIC ALKALINE PHOSPHATASE) ASSAY AND THE OCTEIA™ OSTASE® ASSAY

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The Beckman Coulter Access® Ostase® assay (Beckman Coulter UK Ltd., Oakley Court, Kingsmead Business Park, London Road, High Wycombe, Bucks, UK) is based on the Hybritech® Tandem®-R Ostase method for the quantitative measurement of bone-specific alkaline phosphatase (BAP) in human serum and plasma. The OCTEIA™ Ostase® BAP enzyme immunoassay (IDS Ltd., 10 Didcot Way, Boldon Business Park, Boldon, Tyne and Wear, UK) also shares this antibody technology. The aim of this study was to compare results across these two assays.

126 samples that had BAP routinely measured by the Beckman Access® Ostase® automated immunoassay had aliquots stored at −20 °C for this study. The clinical indication for the initial request in these samples was Paget's disease ($n=79$), osteoporosis ($n=33$), and others/not stated ($n=14$). Samples were thawed, mixed, and spun at 3500 rpm for 5 min prior to analysis using the OCTEIA™ Ostase® assay.

Results of both assays were collated on Microsoft Excel® software and analysed using the Analyse-It® statistical package. Linear regression analysis of Access® v. OCTEIA™ gave an R^2 value of 0.97 and a slope of $y=1.168x+0.1221$.

Conclusions: There is good correlation between the two methods, but a 17% positive bias of the OCTEIA™ assay over the Access® assay. Serial measurement of BAP is usual, e.g. to monitor treatment efficacy, compliance, etc. The bias may therefore be a factor if changing methods is being considered. The good correlation between the two assays suggests that the percentage change across serial measurements within each assay should be comparable.

TP1.17

CRP VARIO IMPLEMENTS VARIABLE RANGE MEASUREMENT OF C-REACTIVE PROTEIN ON AEROSET® and ARCHITECT® c8000

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CRP Vario from Sentinel is a latex-enhanced immunoassay for variable range testing of CRP on the Aeroset/c8000 analysers. Reaction parameters were optimised for blood-sparing high-capacity CRP testing (<5 μl sample) on both instruments.

Concentrations are automatically derived from a 6-level calibration curve. Substituting the top level calibrator from the standard 5–320 mg/L CRP Calibrator Set with a 2.5 mg/L or a 480 mg/L calibrator allows Ultrasensitive (us) and Wide Range (wr) quantitation. Calibrators are traceable to CRM 470. Analytical performance was established using standard protocols.

Detection limit on both instruments was ≤0.1 mg/L and ≤0.2 mg/L for wrCRP and usCRP, respectively. Functional sensitivity (≤20%CV) was ≤0.1 mg/L for usCRP on both analysers, and for wrCRP <0.3 mg/L on Aeroset, and <0.2 mg/L on c8000.

Linearity without dilution was at least up to the last calibration point (160 mg/L usCRP and 480 mg/L wrCRP). No prozone was observed up to 1000 mg/L. A measuring range up to 4800 mg/L is achieved when automatic rerun and 1:10 dilution at CRP levels exceeding the highest calibrator are activated.

Within-run/total imprecision %CVs at target CRP 5–150 mg/L ranged 0.50–1.32/0.76–1.46 on Aeroset and 0.32–1.02/0.49–1.25 on c8000 for usCRP. For wrCRP, at target CRP 5–250 mg/L %CVs ranged 0.76–1.29/1.18–1.92 on Aeroset and 0.77–1.24/1.17–1.91 on c8000.

On Aeroset, accuracy assessment vs. a nephelometric CRP assay generated linear regression parameters slope/intercept/Pearson r 0.996/0.19/0.999 for usCRP and 1.039/0.11/0.999 for wrCRP. Comparison of c8000 vs. Aeroset gave 0.985/0.06/0.999 for usCRP and 0.984/0.30/0.998 for wrCRP.

CRP Vario on Aeroset/c8000 provides for reliable high-capacity CRP testing spanning a 10,000-fold concentration gradient.

TP1.18

RAPID DETECTION OF URINARY TRACT INFECTION – A SCREENING METHOD

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Bacteria are the most common cause of an infection of the urogenital tract (UTI). The presence of leucocytes in urine is an

indicator of the immune response. To discriminate between infection and contamination it is important to quantify the leucocyte count and examine the sediment.

Quantitative counts for bacteria and leucocytes can be simultaneously analysed with a Sysmex Bacsys-40i analyser within 2 min. The technological principle is a fluorescence flow cytometer with semi-conductor laser. A specific fluorescent dye stains nucleic acids within the cells.

The correlation coefficient between the leucocytes determined by BACSYS-40i and cell counting in Fuchs-Rosenthal chamber is 0.979, regression of Passing/Bablok is $y=1.0x+1.0$. Bacteria count analysed with the BACSYS-40i is higher than culture count. If the cut-offs of the analyser were fixed at ≥ 106 and <104 bacteria/μL for positive and negative results, 29 of 39 patients (74.4%) with clearly predominant clinical signs and isolated urinary tract pathogens were recognised. If the defined cut-off of the BACSYS 40i is combined with higher leucocytes count (>20/μL) an additional 9 patients (38/39; 97.4%) were recognised as positive.

Bacteriuria was assessed by semiquantitative culture in 28 samples (71.8%) with ≥ 105 CFU microrganisms/μL urine, the other 11 samples containing ≥ 103 cfu/μL were equivocal positive.

Urine samples can be differentiated by the BACSYS-40i in a few minutes according to the amount of bacteria and leucocytes. 97.4% (38/39) of all patients with clearly predominant clinical signs and isolated pathogens for UTI had positive sample results with the analyser. Compared to the semiquantitative cultures only 28 samples of the patients (71.8%) had ≥ 105 cfu microrganisms/μl urine.

TP1.19

NEW ENZYMATIC CREATININE ASSAY SHOWING STABLE ENDPOINT AND NO INTERFERENCE BY CREATINE AND LIPIDS

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In clinical chemistry different methods are used for the measurement of creatinine. The kinetic Jaffe method is an efficient chemical test for creatinine. Its main disadvantage is the strong interference by bilirubin and its toxicity. The enzymatic UV method is non-toxic but exhibits interference by ammonia. Other enzymatic methods are usable as endpoint assay but show low and unstable signals. Care must be taken for interferences by ascorbate, creatine and proline. Turbidity due to high lipids reduces the range of measurement by high level signal base.

Our test consists of creatininase, creatinase, sarcosineoxidase and a peroxidase-trinder system. The combination of 3-Hydroxy-2,4,6-Triiodo Benzoic acid (HTIB) and 4-Aminoantipyrine (PAP) in TAPS buffer pH 8.1 shows stable signals during measuring time even after incubation at 37 °C for 9 days. We suppress endogenous signals by using catalase in a prereaction until the assay is started with creatininase. Moreover, we introduce a clearing system which eliminates nonspecific signals due to high lipid contents. A combination of detergents solubilises lipids during prereaction. We also use ascorbate oxidase to minimise interferences.

Our novel enzymatic creatinine assay takes along all advantages of endpoint determination by Trinder reactions avoiding low and unstable signals. The reagent can easily be adapted on every clinical chemistry analyzer. It resolves interferences by endogenous creatine (40 mg/dl), proline (10 mg/dl), bilirubin (20 mg/dl) and ascorbate (25 mg/dl). Our lipid clearing system efficiently removes lipids (1500 mg/dl). Measurement range is 0.1–20 mg/dl and method comparison against a commercially available test (Hitachi analyzer) shows significant correlation according to Passing-Bablok (slope 1.04; intercept −0.1 mg/dl, samples 100, $r=0.98$).

TP1.20

POLYCHROMATIC MEASUREMENT ON THE BAYER-1650 ANALYSER ENABLES SIMULTANEOUS MEASUREMENT OF GLUCOSE AND QUANTITATIVE INDICES (HAEMOLYSIS, ICTERUS AND LIPAEMIA) ALL PERFORMED WITHIN THREE MINUTES

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The Bayer ADVIA-1650 analyser has polychromatic measurement capabilities. This allows for simultaneous calculation of glucose and sample indices determination in the same time interval.

Glucose was analysed using the Ecoline-100 kit (glucose-dehydrogenase method; VWR International). Reagent wetting agent is added to rupture erythrocytes if any are present in the heparin plasma sample. The ADVIA-1650 prepares a standard predilution by pipetting 30 μL of sample to 120 μL of saline. From these five-fold dilutions, the analyser pipettes 4 μL diluted sample and 95 μL of modified glucose reagent into the measuring cuvette. If glucose exceeds 60 mmol/L a second predilution is made of 12 μL of prediluted sample and 48 μL of saline. Glucose was measured kinetically. The indices test is an end-point measurement.

Without automatic rerun the glucose assay is linear to 60 mmol/L. With automatic predilution the assay was linear to 300 mmol/L enabling analysis of peritoneal dialysis liquid. Precision: Two levels of BioRad controls: mean 5.28 mmol/L, CV 1.4% and mean 20.6 mmol/L, CV 1.2%. Indices factors were set to provide estimates of haemolysis as free Hb (μmol/L), icterus as bilirubin (μmol/L) and lipaemia in the heparin plasma sample. Haemolysis, icterus and lipaemic interference criteria were set for 24 assays individually. Above the limit haemolytic, icteric or lipaemic is reported.

The ADVIA-1650 provides flexible programming of the assay parameters. This supports the use of the very extended linear range for the glucose assay. The glucose and indices are both reported in 3 min. The quantitative indices assay prevents the reporting of misleading assay results.

TP1.21

A NEW PARTICLE-ENHANCED ASSAY FOR DIRECT IMMUNONEPHELOMETRIC DETERMINATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT)

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The measurement of carbohydrate-deficient transferrin (CDT), a group of transferrin isoforms lacking one or two N-glycans and occurring with high prevalence in individuals with chronic alcohol consumption, is used for laboratory diagnosis of chronic alcohol abuse. After the development of a suitable and specific CDT monoclonal antibody an immunonephelometric assay was established. Here we present a fully automated homogeneous particle agglutination inhibition assay for determination of CDT (N Latex CDT, under-development).

Polystyrene particles coated with a monoclonal antibody against CDT are agglutinated by a second CDT-coated polystyrene particle type. CDT in the sample inhibits the reaction between antibody-coated and CDT-coated particles in a dose-dependent manner. The increase in light scattering is monitored over 12 min, resulting in a total assay time of 18 min using the Dade Behring BN™ Systems. The assay allows a fully automated determination of CDT without any sample pre-treatment.

A sample dilution of 1:5 covers the normal and clinical CDT-range for alcohol abuse. The measuring range of the assay is approximately 20–635 mg/L CDT. Parallel determination of total transferrin allows the calculation of %CDT, which is automatically done by the BN™ Systems. Coefficients of variation (CV) for within-run precision were found between 2.8 and 5.2%, between-run precision yielded CV between 1.6 and 7.6% at CDT levels of 47, 61, 166 and 214 mg/L, respectively. Comparative results from patient samples representative of normal and abnormal levels of CDT showed good agreement between a commercially available assay and N Latex CDT.

N Latex CDT provides a fast, specific and sensitive method for the determination of CDT. This assay will improve and simplify the laboratory diagnosis of chronic alcohol abuse.

TP1.22

EVALUATION OF IQ™-200 AUTOMATED URINE MICROSCOPY ANALYSER

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IQ™-200 is an automated urine microscopy analyser using digital imaging and Automatic Particle Recognition (APR) software to classify urine constituents. Its analytical performance was evaluated in terms of precision, carryover, and the results were compared with manual microscopy. For within-run imprecision two urine pools (high-mean 202 RBC/HPF, low-mean 1 RBC/HPF) were analysed for 10 replicates, for between-run imprecision two quality control pools (high-mean 987 RBC/HPF, low-mean 5 RBC/HPF) were analysed daily for 10 days and results were expressed as CV %. For low and high pools, within run imprecision were 2% and 3%, respectively, between-run imprecision were 3% and 2%, respectively. To assess carryover two pools were prepared, one with low RBC (1 RBC/HPF) and one with high RBC (935 RBC/HPF) and the carryover was calculated less than 1%. For method comparison 150 randomly selected urine samples from different departments of Dokuz Eylul University Hospital were analysed manually and with the IQ™-200. Specimens were judged abnormal if the erythrocyte count exceeded 3 cells/HPF, leukocytes exceeded 4 cells/HPF, and hyaline casts (HC), pathologic cast (PC), renal epithelial cells and crystals equaled or exceeded 1 count/HPF. A good agreement was obtained between the two methods in terms of RBC, WBC count, squamous epithelial cells, renal epithelial cells and crystals ($p > 0.05$), but IQ™-200 improved the detection of HC and PC ($p < 0.05$). Overall, we found that IQ™-200 increased the microscopical sensitivity and resulted in 24% more reportable abnormalities. We concluded that, the similarity of urine constituents seen with IQ™-200 and visual microscopy, the increased microscopical sensitivity and more standardised automated procedure may offer significant benefits to a routine laboratory with high workload.

TP1.23

ROUTINE USE RELATED COMPARISON STUDY OF TWO DIFFERENT PARTICLE-ENHANCED IMMUNONEPHELOMETRIC ASSAYS FOR IGG3 AND IGG4

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The study compared two assays for the determination of human IgG3 and IgG4 [Dade Behring, Marburg (DB) and The Binding Site, Birmingham (TBS)]. Under routine laboratory conditions imprecision, comparability of results, agreement of the sum of the individual subclasses IgG1-4 versus total IgG, as well as on-board stability on a BN™ II analyzer (Dade Behring) were evaluated. Measurements were performed by NGH Sheffield, statistics by DB. Precision was estimated by analyzing two sample pools (high and low) over a period of 18 days with four determinations per day. Comparability of results was evaluated by analysis of 60 routine samples with all methods by means of Passing-Bablok regression. Repeats needed to obtain quantitative results were counted. The agreement of the sum of the individual IgG-subclasses 1–4 (SumIgG1–IgG4) with total IgG was calculated. The on-board stability of the reagents in opened vials was monitored for 1 week using control measurements.

Imprecision (total CV; %) for the low and high pool, respectively, was 5.2/5.8 (DB), 5.8/8.0 (TBS) for IgG3, and 5.4/5.8 (DB), 9.5/8.8 (TBS) for IgG4. For routine samples analyzed the re-testing rate was zero for DB and 7% for TBS. The DB reagents were stable over the whole 5 day period, but the TBS reagents only for 2 days. Regression analysis between SumIgG1 and IgG4 versus total IgG yielded median deviation results of +1.8% for DB and −9.0% for TBS. Individual comparisons between methods were acceptable but showed significant biases due to different standardization.

Overall, the DB methods showed better precision, no repeat testings, excellent agreement between total IgG and sum of the subclasses and a superior on-board stability.

TP1.24

MULTI-CENTER EVALUATION OF THE URISYS® 1800 URINE ANALYSER

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Urisys® 1800, a new urine analyser for multiple reagent test strips (Combur10 Test® M; Combur9 Test® M) underwent pre-market evaluation at several international sites prior to its release. Benefits of the new system are an improved illumination of the test strip area, touch screen display, a clearly arranged user-interface and continuous test strip loading.
The study objectives focused on performance and practicability of the new analyser.
Methods: The assessment included intra- and inter-assay precision, recovery of controls, calibration stability, colour rating and result comparison with different methods. Practicability in different working scenarios under routine simulating conditions was evaluated by a detailed questionnaire comparing Urisys® 1800 with the currently used routine instrument. Reliability and robustness were checked during the whole evaluation phase.
Results: 99% of results met pre-defined specifications. Calibration was stable for at least 4 weeks. Daily measurements of controls with normal and abnormal concentration confirmed the stability and accuracy of the analyser. Results from commercial control materials were within claimed ranges. Intra- and inter-assay CV's for controls and human samples ranged from 0.2–5.7% indicating high precision. Glucose and protein determinations correlated well with clinical chemistry analysers, urine colour determinations widely agreed with visual rating (>75%). Lower concordance to flow cytometry and microscopic analysis was attributable to limited comparability of methods. The quality of experimental data was equal between the evaluation sites emphasising reproducibility and robustness of system production. Practicability was rated as highly reliable, robust and easy in off-line and on-line usage.
Conclusion: The performance of Urisys® 1800 exceeded our expectations for use in a small to medium size laboratory. Higher throughput and convenient operation improve user friendliness compared to previously available analysers.

TP1.25

EVALUATION OF THE NEW ACCESS® GI MONITOR ASSAY FOR THE QUANTITATIVE DETERMINATION OF CA 19-9 ANTIGEN LEVELS

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The new ACCESS® GI MONITOR (Beckman Coulter), a paramagnetic particle chemiluminescent method, was evaluated and compared to two other immunoassays.
Two commercial Bio-Rad controls (~8 and ~28 U/ml) and one commercial Probioqual control (~120 U/ml) were used to determine precision, according to NCCLS Guidelines. Linearity upon dilution was tested on 5 elevated samples. The reference range was evaluated in 50 apparently healthy persons. Deming regression, Bland and Altman's difference plot and concordance analysis were used to compare ACCESS® GI MONITOR with Kryptor CA 19-9 and Elecsys CA 19-9 assays in 100 serum samples selected to span the dynamic range of the assay (0.8–2000 U/ml).
Intra-assay precision was below 5% CV and inter-assay precision ranged from 3.7% to 8.8% CV. All diluted samples produced a linear response. The 95th, 97.5th percentiles and the maximum value of the reference population were 20.1, 29.35 and 32.9 U/ml, in concordance with the commonly used 35 U/ml cut-point. The Deming regression slopes were 1.48 ($r=0.9290$) and 1.14 ($r=0.9422$) for ACCESS vs. Kryptor and Elecsys across the assay dynamic range. The mean % difference between ACCESS and Kryptor was 19.4% and 8.2% between ACCESS and Elecsys.
In conclusion, ACCESS® GI MONITOR demonstrates reliable performance and may provide useful information in cancer patients' evaluation.

TP1.26

LITHIUM HEPARIN PLASMA CAN BE USED FOR PROTEIN ELECTROPHORESIS AND PARAPROTEIN IDENTIFICATION

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Introduction: Lithium-heparin plasma is the most commonly used sample type in many hospitals but is not suitable for protein electrophoresis due to the presence of fibrinogen, which can potentially mask a paraprotein band. Even serum samples can have a fibrinogen band present, for example in patients on anticoagulation therapy. It was our aim to determine whether ethanol treatment can remove the fibrinogen band in lithium-heparin samples without affecting the integrity of other proteins.
Method: A lithium-heparin sample from a patient with IgGλ myeloma, IgGκ myeloma, IgAλ myeloma, IgAκ myeloma, a non-specific polyclonal increase and a serum control was treated with ethanol of varying concentrations prior to protein electrophoresis. The effect of the ethanol treatment on fibrinogen bands was assessed. Immunofixation electrophoresis was also undertaken to investigate the effect of ethanol treatment on immunoglobulin and light chain identification. Nephelometry was undertaken to investigate whether ethanol treatment affected the quantification of IgG levels and densitometric evaluation of proteins after electrophoresis was used to study whether the treatment affected the other proteins.
Results: 120 mL/L of ethanol significantly removed the fibrinogen bands in all cases compared to untreated controls. Ethanol treatment did not affect the detection of immunoglobulins, light chains or any other protein.
Conclusion: Lithium-heparin plasma can be used for protein electrophoresis for the detection of paraproteins after ethanol treatment. This has implications in terms of saving costs and prevents the need for requesting another serum sample form patients. This may be one step towards a universal sample for all tests.

TP1.27

EVALUATION OF A NEW AUTOMATED INTRINSIC FACTOR ANTIBODIES ASSAY

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An automated assay for Intrinsic Factor Antibodies (IFA) is available on the ACCESS 2 analyser from Beckman Coulter. It is a two-step chemiluminescent method based on competition between IFA and anti-intrinsic factor MAb complexes for the cobalamin binding site on alkaline phosphatase-linked hog intrinsic factor.
We evaluated assay linearity and reproducibility, we compared results with a conventional IFA enzyme immunoassay (Medizym) and we studied and eliminated assay interference by high serum vitamin B12 levels.
The assay displayed good linearity across a wide dynamic range with good sensitivity at low antibody titres. Within assay CVs ($n=10$) were 3.1% and 7.6% for ACCESS controls, and 4.4%, 2.1% and 2.6% for patient sera (negative, low titre positive and high titre positive). Between assay CVs were 1.8% and 2.7% for the ACCESS controls, and 2.6%, 6.7% and 3.4% for the sera.
ACCESS 2 and Medizym ELISA (Medipan Diagnostics) IFA assays compared well with a similar interpretation in 57 out of 63 (83%). Of the remaining 12 samples, 7 were positive by Medizym and negative by ACCESS, 2 were positive by ACCESS and negative by Medizym and the remaining 3 were equivocal by one method, and negative by the other.
The manufacturer states that levels of free cobalamin above 444 pg/ml may cause false positive results. We noted that IFA negative patient sera became positive after an addition of some 1000 pg/ml to 2500 pg/ml of cyanocobalamin, depending on the cobalamin binding capacity of the sera. We eliminated false positive results using an albumin-coated charcoal pre-treatment step which was effective in sera with at least 10,000 pg/ml of added cyanocobalamin.

TP1.28

CAN POTASSIUM VALUES BE CORRECTED IN HAEMOLYSED SAMPLES?

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It has been advocated that measuring the haemoglobin concentration in a haemolysed sample can be used to correct the potassium concentration. However, estimation of the degree of potassium adjustment has not been validated with patient derived data. This study aimed to ascertain the relationship between haemolysis and potassium increment using patient samples, and to compare it with data from a simulated model.
Thirty-five hospital patients (M,F) in whom haemolysed samples were repeated within 24 h were included in the study. The difference between the potassium of the haemolysed and non-haemolysed repeat sample was correlated with the serum haemolysis index measured on a Beckman Synchron LX20PRO. The patient derived data were compared with a simulated model in which potassium increment in artificially induced haemolysed serum was correlated with a spectrophotometric serum haemoglobin concentration.
The derived linear relationship in the patient samples was not significantly different from the simulated model ($p=0.73$) showing a potassium increase of approximately 0.15 mmol/L for each increment in haemolysis index. However, Bland and Altman statistics showed the variability (95% limit of agreement) in predicted potassium concentration to be ± 0.73 mmol/L.
The variability indicates that in haemolysed serum, two patients with identical corrected potassium results could have true values differing by 1.46 mmol/L. In conclusion, the relationship does not obviate the need for a repeat specimen, but may be a useful guide to its urgency.

TP1.29

IMPRECISION AND INACCURACY ASSESMENT OF A KINETIC INTERACTION OF MICROPARTICLES (KIMS) IMMUNOASSAY OF PHENYTOIN

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Objective: To validate the ONLINE®TDM inmunoassay based on KIMS technology implemented in a Roche MODULAR P platform.
Methods: Imprecision was evaluated by means of Roche ONLINE®TDM controls (three levels). Within-run (WR) imprecision was performed running each control level along three days ($n=60$). Between-run (BR) imprecision was assessed for 20 consecutive days ($n=20$). Inaccuracy was established by parallel measurements in 95 patient's serum samples collected from TDM department and stored at -20 °C. Phenytoin concentrations ranged from 5 to 35 μg/ml. The comparison methods were FPIA (AxSym-Abbot Diagnostics) and dry chemistry (Vitros950-Johnson&Johnson CD). Limit of detection was evaluated with a serum sample free of phenytoin.
Results: Phenytoin mean values were 6.55, 13.40, and 25.35 μg/ml for each control level. WR CVs were 3.2%, 4.1%, and 3.2%, and BR CVs were 4.7%, 3.6%, and 4.2% for the three control levels studied. Correlation coefficients were 0.995 versus Vitros950 and 0.991 versus AxSym. Deming regression analysis yielded a slope 1.055 (95% C.I.: 0.875–1.342) and an intercept of 0.060 (95% C.I.: −0.025–0.142) using Vitros950 as a reference, and a slope 1.055 (95% C.I.: 0.875–1.342) and an intercept of 0.060 (95% C.I.: −0.025–0.142) when AxSym was the comparative method. Passing–Bablock agreement test gave similar results.
Limit of detection was 0.3 μg/ml.
Conclusions: Good precision results at low, medium and high levels of phenytoin met the analytic goals proposed by NACB. Statistical tests of comparative analysis showed marked correlations with two distinct technologies (dry chemistry and FPIA).

TP1.30

IDENTIFICATION AND CHARACTERIZATION OF CHEMICAL SPECIES AND METABOLITES OF SELENIUM IN BLOOD, BREATH, URINE AND NUTRITIONAL SUPPLEMENTS

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The bioavailability and toxicity of Selenium are highly dependent on the chemical speciation of the element at the point of ingestion, and

also upon the metabolic pathways of the various species. Toxicity and nutrition vary by species. Whilst robust analytical methods for measurement of total Selenium exist for a variety of matrices, methods are required that can separate, quantify and characterise individual species of varying nutritional value or toxicity.
Although inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive technique for elemental analysis, and can be used in conjunction with liquid chromatography to separate species, it fails to provide any structural information. Only comparing their retention times with standards, which are not always readily available, can identify individual species. Triple Quadrupole Linear Ion Trap Mass Spectrometry (TQ-LIT-MS), combined with liquid chromatography offers both quantitative and qualitative information of individual species. The identification of the individual compound can be determined during a liquid chromatography run, including identification of species and metabolites that are unknown and for which standards are not available. The technique is therefore useful as both a diagnostic tool, to quantify and confirm the presence of each species in clinical samples and also to examine the structure of previously unknown chemical species in various matrices.
We have studied the use of TQ-LIT-MS for measurement and characterisation of individual Selenium chemical species and metabolites in clinical matrices, including blood, breath and urine and also nutritional supplements. We will show results from our studies and outline significance of our results towards clinical chemistry.

TP1.31

A HOMOGENEOUS, HIGH-THROUGHPUT SCREENING METHOD FOR DETECTION OF CALCIUM DEPENDENT PROTEIN OLIGOMERISATION

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High-throughput screening is the process of testing a large number of diverse chemical structures against targets to identify hits. In order to screen 100,000 molecules per day the assays should be fast, simple and highly automated. Homogeneous mix-and-measure assay format goes well together with these requirements.
We have developed a homogeneous high-throughput drug screening method for detection of protein oligomerisation based on time-resolved fluorescence resonance energy transfer (TR-FRET). As a model target system, we have used an intracellular signaling complex. The complex, which contains few protein monomers, is calcium dependent.
In the assay, peptides specific for the protein complex were labeled with prompt Alexa700 fluorophore (Molecular Probes, USA) and with long-lifetime fluorescent europium chelate. Upon formation of the protein complex, the labeled peptides are brought into close proximity, which allows energy transfer between the donor and acceptor labels. When europium is excited at its maximum excitation wavelength (340 nm), long-lived acceptor emission is detected at 720 nm, at which wavelength background fluorescence is minimal.
With the assay, we could detect calcium dependent formation of the protein complex in a homogeneous manner. The observed signal-to-background ratios were about 20. Signal level was decreased to the background level after removing calcium from the assay solution, which proves that the formation of the protein complex is reversible. The assay does not include washing steps, it is fast, easy and it allows us to screen compound libraries for candidate drugs that either inhibit or enhance oligomerisation.

TP1.32

COMPARISON OF ESTIMATED GFR WITH CREATININE CLEARANCE IN ROUTINE PRACTICE

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Glomerular filtration rate (GFR) is related inversely (non-linearly) to serum creatinine. Accordingly, a measure of GFR is advised for people with chronic kidney disease (CKD) and those at risk for CKD. However, such GFR estimates are supposed to be less precise above 60 ml/min/1.73 m2.
We estimated GFR using the MDRD equation in 100 patients undergoing a creatinine clearance test (CCT) using a kinetic alkaline picrate assay. Absolute and relative difference plots were performed and the mean bias and range of differences were calculated for all stages of CKD.
The mean GFRs ranged from 7–178 ml/min/1.73 m2 with 22, 22, 39, 12 and 4 patients having a mean GFR consistent with CKD stages 1, 2, 3, 4 and 5, respectively. Compared to the mean GFR, the estimated GFR had a mean bias of −50%, −41%, −36%, −37% and −40% in CKD stages 1, 2, 3, 4 and 5, respectively. The ranges of the relative differences were 82%, 86%, 97%, 88% and 96%, respectively, in CKD stage 1, 2, 3, 4 and 5.
As expected, the MDRD is negatively biased compared to the CCT and the absolute difference increased with increasing mean GFR. There was no evidence to suggest that the GFR estimates were less precise above 60 ml/min/1.73 m2, but there may be a difference in the mean bias above 90 ml/min/1.73 m2. As the non-diseased total coefficient of variation for CCT is approximately 25%, any such bias is unlikely to be clinically significant. Accordingly, if CCT results are acceptable to be reported across all stages of CKD, then we believe that MDRD GFR estimates should be reported across all stages of CKD.

TP1.33

AMPEROMETRIC IMMUNOSENSOR FOR RAPID DETECTION OF *VIBRIO CHOLERAE* 01 ANTIGENS

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Cholera is an important acute diarrheal disease presenting with severe watery diarrhea, dehydration, acidosis, circulatory failure and death. Rapid and early detection of the causative organism, *Vibrio cholerae* O1, is the key role in controlling this disease.

In this study, an amperometric immunosensor on screen-printed carbon paste electrode for *V. cholerae* O1 antigen detection was developed using polyvalent anti-*V. cholerae* O1 as capture antibody, mouse monoclonal anti-*V. cholerae* O1 as primary antibody, goat-anti mouse Ig conjugated with alkaline phosphatase as secondary antibody. Para-aminophenyl phosphate was used as substrate for detection of alkaline phosphatase activity by amperometric measurement. The developed method was compared to enzyme-linked immunosorbent assay (ELISA) using the same set of antibodies.
The optimal condition for the immunosensor was 20 ng/10 ml of IgG fraction of polyvalent anti-*V. cholerae* O1, 500 ng/10 ml of mouse monoclonal anti-*V. cholerae* O1 and 1:2000 dilution of goat anti-mouse Ig-conjugated with alkaline phosphatase. For standard ELISA technique, the lowest detectable numbers of El Tor *V. cholerae* O1 serotype Ogawa (ETO) in alkaline peptone water (APW) and mixture of coliforms were both 1×108 CFU/ml, while such numbers for Inaba serotype were 1×106 CFU/ml and 2.5×106 CFU/ml. The developed immunosensor gave the same or better results for the lowest detectable numbers of ETO, both in APW and mixture of four coliforms which were 2.5×106 and 1×108 CFU/ml, while the numbers for Inaba serotype in the same manner were 7.5×105 and 2.5×106 CFU/ml, respectively. All 13 clinical isolates of V. cholerae O1 gave the positive results. No cross-reaction was observed among 39 isolates of both gram positive and gramnegative bacteria tested.

TP1.34

NON-CAERULOPLASMIN BOUND COPPER IN CLINICAL PRACTICE

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Copper is an essential co-factor for many enzymes including cytochromes, but is toxic in its unbound form. The vast majority of serum copper is transported bound to caeruloplasmin; the rest is attached to albumin, transcuprein and copper-amino acid complexes. Calculation of non-caeruloplasmin-bound copper (NCC) may be used to identify patients with Wilson's disease (an autosomal recessive disorder with a frequency of 1:30,000–100,000 live births) and other copper disorders due to variation in serum caeruloplasmin concentrations. However, there is little data about its effectiveness or robustness in clinical practice.
We reviewed the copper, caeruloplasmin and NCC results for 338 individual patients who had both copper and caeruloplasmin requested. No patient was subsequently diagnosed as having Wilson's disease.
The copper and caeruloplasmin results ranged (median) from 7–41 (17) μmol/L and 180–730 mg/L (335), respectively. The relationship between copper and caeruloplasmin was curvilinear ($r^2=0.85$) but approximated to linear ($r^2=0.84$). The NCC ranged (median) from −7.8 to 12.0 (1.6) μmol/L. 68 NCC results were negative and 161 results were >1.6 μmol/L.
A negative NCC is not theoretically possible as copper is also bound to albumin, transcuprein and copper-amino acid complexes but was yet found in 20.1% of patients. 1.6 μmol/L is considered to be the upper reference interval and yet 47.6% of results were above this cut-off. No standardised reference method exists for caeruloplasmin and immunological methods employed for caeruloplasmin cross-react with apocaeruloplasmin. This together with method related differences including bias, precision and specificity may have a larger effect on NCC than previously thought. We believe that these findings require further investigation to prove the effectiveness and robustness of NCC in clinical practice.

TP1.35

COMPARISON OF IQ 200 SYSTEM AND ROUTINE METHODS IN URINE ANALYSES

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Aim: A routine urine analysis, especially sediment is one of the last subjective routine methods in medical biochemistry laboratories. There are several automated systems for urine analysis helping reading tests strips and also sediment. IQ 200 SYSTEM is an integrated fully automated system for routine urine analysis. The aim of our study was to compare routine urine analysis performed by laboratory technicians and the automated analyser.
Methods: Between-day imprecision was determined in 38 positive control urine. Within-run imprecision was determined in 30 consecutive measurements in positive control urine. Correlation of the urine analyser and subjective evaluation by eye (test strips) and microscope (sediment) were performed using 300 unselected urine samples. Spearman's coefficient of rank correlation (rs) was calculated for the variables being monitored.
Results: Between-day imprecision CV=7.0% and within-run CV=4.4%. Spearman's correlation coefficients between results obtained from analyser and subjective estimation of test strips were calculated for glucose (rs=0.74), proteins (rs=0.62), leukocytes (rs=0.77) and erythrocytes (rs=0.88). Correlation between sediment analyses obtained from IQ200 and microscopic evaluation was estimated. Spearman's correlation coefficients were as follows: leukocytes (rs=0.52), erythrocytes (rs=0.52), cylinders (rs=0.43), squamous epithelium cells (rs=0.59).
All correlations coefficients calculated were significant ($p<0.001$).
Conclusions: There is a moderate to good degree of relationship between results obtained by the analyser and human eye in routine urine analyses. The traditional method for urine analysis can be successfully substituted with automated urine analysis system which has very high accuracy and reproducibility with regard to CV.

TP1.36

PARATHYROID HORMONE IN ROUTINE CLINICAL PRACTICE WITH THE ADVIA CENTAUR

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Measurement of PTH is important in the evaluation of patients with disorders of calcium metabolism. We examined the difference between EDTA plasma and serum specimens in routine clinical

practice as opposed to controlled laboratory conditions with the Advia Centaur.

Serum and potassium EDTA blood samples were collected immediately before patients underwent haemodialysis. There was no extra emphasis placed on time requirements before or during the study. Aliquots of plasma and serum were promptly frozen after centrifugation.

The Deming regression equation was Serum=0.8927 EDTA-0.447 and the 95% confidence intervals for the slope and intercept were 0.8558 to 0.9296 and −1.6361 to 0.7421. The slope was significantly different from 1.0 and intercept was not significantly different from zero. The mean difference (([EDTA]−[serum])/2)) was 13.8%, and the S.D. was 5.8%. Accordingly, the 95% confidence interval for the data was 2.2% to 25.4%. No significant effect of time to freezing was noted.

We have observed statistically significant differences in the PTH concentration between serum and EDTA plasma in routine clinical practice. Our results are in general agreement with those of recent investigations examining controlled non-routine specimens that employed different analytical systems. We believe that our results add to the current literature by verifying that EDTA specimens produce higher results than serum in routine clinical practice. Our data showed that a large inter-individual difference exists for PTH concentrations when different specimen types are used in a routine clinical setting and that this difference seems to be independent of the time to separation and the PTH concentration.

TP1.37

DEVELOPMENT OF A MICROSPHERE BASED IMMUNOASSAY FOR THE QUANTIFICATION OF COMPLEMENT FACTORS H AND I

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Complement is a central component of innate immunity and provides a multifactorial response to invading pathogens. The importance of complement is demonstrated by the pathology associated with deficiencies of complement proteins and their regulators.

Complement deficiencies are severely under-investigated. This has been highlighted recently in studies that correlate deficiency of the complement regulators factor H (fH) and factor I (fI) with atypical haemolytic uraemic syndrome and membranoproliferative glomerulonephritis.

The radial-immunodiffusion (RID) and ELISA methods used in the laboratory for complement protein quantification have many inherent limitations and can only facilitate the quantification of one complement protein at a time.

Recent studies have demonstrated that microsphere-based immunoassays can be multiplexed to provide the simultaneous analysis of many analytes.

The aim of this study was to develop and optimise a microsphere-based immunoassay for the quantification of fH and fI. We used 50 anonymised patient samples received in the laboratory for the investigation of complement to compare the developed method with the existing RID assay. The developed method exhibited comparable sensitivity and specificity. This indicates that the microsphere-based platform of analysis is amenable to the multiplexed analysis of a complement profile.

This platform of analysis will greatly improve the investigation of complement deficiency within the laboratory, thus facilitating the fast, simple quantification of a complement profile in one small aliquot of patient serum.

TP1.38

EVALUATION OF A NEW CAPILLARY ELECTROPHORESIS IMMUNOTYPING METHOD FOR THE CHARACTERISATION OF MONOCLONAL PROTEINS IN SERUM

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A new automated method for characterising monoclonal proteins by capillary electrophoresis using an immunosubtraction technique has been launched for use on the Sebia Capillarys system. The system was evaluated for ease of use and ability to correctly identify abnormal paraprotein bands in both patient and EQAS samples. Performance was compared with a semi-automated agarose immunofixation technique (Sebia).

Samples were of varying paraprotein types and concentrations (from faint discrete bands, up to a monoclonal protein of 75 g/L).

Five EQAS and 29 of 38 patient samples analysed were correctly immunotyped by both agarose immunofixation and capillary electrophoresis immunotyping methods. All paraprotein bands with a mass of greater than 5 g/L were successfully immunotyped on both systems.

Seven of the 38 patient samples contained faint discrete paraprotein bands (<5 g/L), which could be identified by agarose immunofixation but not by capillary electrophoresis immunotyping. The capillary electrophoresis immunotyping method employs antibodies, which will remove both polyclonal and monoclonal immunoglobulin fractions from the serum. As IgG typically forms the major immunoglobulin component of the gamma region, it was most difficult to detect a faint discrete band of IgG-containing paraprotein superimposed on polyclonal IgG.

Two very faint discrete bands visualised by serum electrophoresis could not be typed by either the agarose immunofixation or capillary electrophoresis techniques.

The Sebia Capillarys Immunotyping method offers a simple and fast, fully automated system for characterizing paraproteins above 5 g/L. Introduction into routine clinical practice would depend on laboratory workload with potential labour saving benefits. However, a supporting technique e.g. agarose immunofixation would still be necessary to immunotype faint discrete paraprotein bands and heavy chains D and E.

TP1.39

A SENSITIVE AND PRECISE AUTOMATED ASSAY FOR THE DETERMINATION OF SERUM PARC

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PARC (CCL18) is a newly-described biomarker of Gaucher disease. Although it shows promise in the research environment, it has not yet made the transition to routine clinical use. Reagents and standards for the estimation of PARC concentration are available from R&D Systems Inc., Minneapolis, USA. Their "DuoSet" reagents are designed for use as components of an ELISA-based immunoassay. The manufacturer supplies a protocol with recommended reagent concentrations and timings for this assay. However, the assay is imprecise and has a narrow working range. Additionally, samples require multiple pre-dilution steps before analysis. This makes the procedure difficult to automate and adds to between-assay variability (CV 15–25%). Sequential PARC measurements may be used to assess the effectiveness of enzyme therapy. Therefore, a precise and reproducible assay is essential.

We have used the R&D Systems reagents to develop an in-house time resolved fluorescence immunoassay (DELFIA assay). This assay has a much wider working range (15–2000 ng/ml). This allows samples to be analysed with only a single pre-dilution step. The assay has good within-batch imprecision (CV<4% at normal and elevated PARC concentrations) and between-batch imprecision (CV<8% at normal and elevated PARC concentrations). All stages of the assay have been automated on the AutoDelfia analyser (Perkin-Elmer).

Linearity on dilution, interfering factors, analyte recovery and sample stability have been studied and shown to be acceptable for a routine Clinical Laboratory assay. This assay will form part of the routine monitoring of patients with Gaucher's disease in our hospital.

TP1.40

PLASMA 5-AMINOLEVULINIC ACID (ALA) IN THE DIAGNOSIS AND FOLLOW UP OF PORPHYRIA

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Currently, there is no laboratory in the NHS that offers an assay for measurement of plasma ALA. The main indications for measuring plasma ALA are in patients with renal failure. Chronic renal failure is also a known complication of porphyria. Therefore, urinary ALA and PBG cannot be monitored for the diagnosis and follow up in renal failure patients with acute porphyria. Furthermore, plasma ALA is a useful marker of lead exposure as it is increased due to inhibition of ALA dehydratase.

There are few published methods available for measuring plasma ALA (1) enzymatic based on conversion of ALA to uroporphyrin (2) spectrofluorimetry based on derivatisation of ALA following separation by cation exchange and (3) HPLC method using fluorescence detection following derivatisation.

We have modified an HPLC method (Tomokuni et al., Clin Chem 1993; 39: 169) that involved precolumn derivatisation of ALA with acetylacetone and formaldehyde and HPLC using fluorescence detection. The fluorescence was measured at an excitation wavelength of 370 nm and an emission wavelength of 460 nm. The method was linear from 50–1000 μg/L with a detection limit of about 20 μg/L. Within and between assay variation at 200 μg/L was ~7% ($n=5$).

Plasma samples from 30 patients with an acute porphyria (22 AIP, 5 VP, 3 HCP) and 10 non-porphyric patients were analysed. Plasma ALA concentration ranged <20 to 470 μg/L and was highest in those patients recently diagnosed with an acute porphyria (3/30) and who had repeated attacks of acute porphyria (4/30). Patients with latent porphyria had the lowest levels that ranged from 20–50 μg/L. Non-porphyric patients had levels <20 μg/L.

TP1.41

ANALYTICAL AND CLINICAL EVALUATION OF A NEW METHOD FOR THE AUTOMATIC IDENTIFICATION OF BIPHASIC WAVEFORM ON APTT ANALYSIS

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The biphasic transmittance waveform on routinely activated partial thromboplastin time (APTT) is consistent with a diagnosis of a non-overt disseminated intravascular coagulation (DIC) or sepsis. Until now only one instrument possess this detection capability.

We employed a new analyzer, Amax Destiny by Trinity Biotech plc (Ireland), that can obtain a profile of absorbance (rather than transmittance) at 405 nm during the coagulation process. The software was modified by us with a view to setting up a new, so-called Biphasic, test based on the APTT protocol that measures the increase in absorbance mU 18 seconds after the addition of calcium chloride. In a group of 212 healthy donors, this test had a mean value of 8.2 mUAbs and an S.D. of 7.2. The upper normal value was identified at 30. The between-run imprecision (frozen plasma pool) at a mean value of 12.5 was CV 14.2%, and CV 7.8% at 45. In the setting of General Intensive Care Unit in the University Hospital of Padova (5 units, 60 beds) we tested all patients on the day of admission, and also during treatment, over a period of 1 month. In 317 patients (1,902 tests) findings were positive (>30 mUAbs) in 373 tests (19,6%). The clinical conditions associated with a positive test included: severe sepsis, DIC, infections, pancreatitis.

The test, which is simple to perform, low cost and quantitative, can be transmitted to the LIS in a numeric format, conforms to the validation rules for results and can be monitored with a standard quality control program.

TP1.42

EXPERIENCE WITH THE DADE BEHRING DIMENSION HbA1C ASSAY – SOME HAEMOGLOBINOPATHY PROBLEMS SOLVED, BUT SOME REMAIN

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All samples received for HbA1c analysis in the Clinical Biochemistry department at Addenbrooke's were until recently analysed on a Tosoh 2.2 analyser, this included samples from patients with haemoglobinopathies. The Dade Behring Dimension

HbA1C immunoassay method was evaluated as an alternative method for measuring HbA1c in all patients with different haemoglobinopathies.
Samples from 58 diabetic patients with haemoglobinopathies were analysed under this new system. A summary of our findings are: HbS comparable on both, HbD comparable on both, high HbF comparable on both, HbE lower on Tosoh, HbAO Arab lower on Tosoh, HbQ India lower on Tosoh and HbJ lower on Tosoh.
As expected, where a discrepancy between the two methods was found, the Tosoh 2.2 underestimated the HbA1c value compared to the Dade Behring method. Of the above patients, nine had more than one sample sent in the year. For those patients, each sample showed a consistent relationship between the results from the two methods.
Following an evaluation to ensure that routine samples gave comparable results, the decision was taken to analyse all variant haemoglobin samples on the Dade Behring Dimension R×L system. This result is then reported with a comment stating "variant haemoglobin measured using immunoassay to remove possible interference caused by known variant".
Although this strategy has successfully addressed one of the problems with diabetic haemoglobinopathy patients, others remain. HbA1c results on these patients are now similar to DCCT aligned results but it should be noted the original DCCT study excluded haemoglobinopathy patients. Further work on this group of patients is needed to investigate the influence of cell turnover rates on the measured HbA1c.

TP1.43

EFFECTS OF PROCESSING AND STORAGE CONDITIONS ON CSF AMYLOID β (1–42) AND TAU CONCENTRATIONS: IMPLICATIONS FOR USE IN CLINICAL PRACTICE

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Background: Reported concentrations of amyloid-β(1–42) (Aβ42) and tau in cerebrospinal fluid (CSF) differ among reports. We investigated the effects of storage temperature, repeated freeze/thaw cycles, and centrifugation on the concentrations of Aβ42 and tau in CSF.
Methods: Stability of samples stored at −80 °C was determined using an accelerated stability testing protocol according to the Arrhenius equation. Aβ42 and tau concentrations were measured in CSF samples stored at 4, 18, 37, and −80 °C. Relative CSF concentrations (%) of the biomarkers after one freeze/thaw cycle were compared with two, three, four, five, and six freeze/thaw cycles. In addition, relative Aβ42 and tau concentrations in samples not centrifuged were compared to samples centrifuged after 1, 4, 48, and 72 h.
Results: Aβ42 and tau concentrations were stable in CSF when stored for a long period at −80 °C. CSF Aβ42 decreased with 20% during the first two days at 4, 18, and 37 °C compared with −80 °C. CSF tau decreased after storage for 12 days at 37 °C. After three freeze/thaw cycles CSF Aβ42 decreased 20%. CSF tau was stable during six freeze/thaw cycles. Centrifugation did not influence the biomarker concentrations.
Conclusions: Repeated freeze/thaw cycles and storage at 4, 18, and 37 °C influence the quantitative result of the Aβ42 test. Preferably, samples should be stored at −80 °C immediately after collection.

TP1.44

EU(III) NANOPARTICLE BASED SENSITIVE IMMUNOASSAY FOR THE DETECTION OF *LISTERIA* SPP

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Nanoparticle labels have successfully been used in small molecule analyte immunoassays to enhance the assay sensitivity. The increase in sensitivity not only enables the detection of lower concentrations but it also allows the development of very rapid assays. Bacterial detection from food or environmental samples is usually time consuming and laborious. For example, current *Listeria* detection methods may last 2 days to perform. Rapid detection of pathogenic *Listeria monocytogenes* is important because the bacterium can cause serious disease, listeriosis, especially to pregnant women, neonates, the elderly and the immunocompromised.
The standard procedure in *Listeria* analysis is to increase the bacterial numbers by cultivating the samples. The bacterial enrichment step could be shortened significantly by employing a sensitive *Listeria* assay which can detect the bacteria reliably in lower concentrations than present assays. Two-site immunoassay sensitivity can substantially be improved by utilising europium(III) chelate containing nanoparticles as tracers. Each 107 nm nanoparticle contains over 30,000 europium(III) chelates which enhances the specific activity of the label.
The sensitive heterogenous immunoassay developed for *Listeria* spp. utilises monoclonal antibody coated Eu(III) nanoparticle tracers and can be conducted in one-step or two-step formats. One-step assay is notably faster (15 min) and simpler to execute having sensitivity below 500 cfu/ml. However, the two-step assay sensitivity clearly exceeds the one-step assay resulting in sensitivity below 50 cfu/ml. Food and environmental samples were measured against a commercial *Listeria monocytogenes* immunoassay with excellent correlation. The developed sensitive assay enables shorter sample enrichment times and, therefore, faster analysis of *Listeria* spp.

TP1.45

COMPARATIVE MICROANALYSIS OF TEAR PROTEINS: AN EXPERIMENTAL MODEL

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Alteration in tear protein composition may play a significant role in the development of different pathological conditions therefore a sensitive analysis of it may be a useful marker of an underlying

disease. There are only a few studies in the literature with reference to changes of tear protein content focusing on both quantitative levels and protein pattern data. The purpose of our study was to establish a model system for a sensitive comparative microanalysis of tear proteins obtained under experimental conditions.
Two groups of animals with and without pre-treatment with a somatostatin analogue were compared. Tear samples from rats were obtained by a standardised microcapillary technique. From each animal both unstimulated (basal) and electrically stimulated (reflex) tears were simultaneously collected. Protein contents were measured on the individual samples by the Bradford dye binding assay. Tear protein patterns were analysed by SDS–PAGE and two dimensional electrophoresis combined with silver intensification. For identification of individual tear proteins anti-IgA and anti-lysosyme antibodies were used after transfer of the separated proteins onto nitrocellulose membrane. Immunoblots were developed by Ni-DAB+silver intensification and also enhanced chemiluminescence.
Our results showed a significant increase in the volume of the reflex samples compared to that of the basal secretion. Protein concentrations decreased after electric stimulation and did not change significantly in the pre-treated group. However, the total protein output during the 20-min secretion period increased in both stimulated groups. We could not find any difference either in the protein patterns or in the appearance of IgA and lysosyme between the basal and reflex tears when samples were adjusted to protein contents.

TP1.46

BIOSITE TRIAGE® BNP ON THE BECKMAN COULTER ACCESS 2

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The original Triage® B-Type Natriuretic Peptide (BNP) Test (Biosite Incorporated, San Diego) utilised the Triage Meter for BNP determination at the point-of-care. Triage BNP was subsequently adapted for the Beckman Coulter Access 2 (BCA2) to enable laboratory-based analysis using the same two-site ("sandwich") immunoassay. Triage BNP on the BCA2 was designed and standardised for interchangeable use with the original assay over the entire analytical range of 5–5000 ng/L.
To assess analytical performance of Triage BNP on the BCA2, this evaluation began with the NCCLS EP10 Preliminary Evaluation of Quantitative Methods using control materials designed for the original Triage BNP. Bias and imprecision were acceptable. Bias was $<-1.5\%$ and within-run and total CV's were $<2.0\%$ at BNP levels of 90, 1100 and 2200 ng/L. Linearity, carryover and drift were acceptable. The t values for slope, intercept, % carryover, nonlinearity and drift were $<\pm 2.0$ (acceptable range $<\pm 4.6$).
Additional assessments of imprecision over 15–20 days were acceptable with a total CV of 2.0, 1.1 and 1.3% at BNP levels of 90, 400 and 2100 ng/L.
To assess analytical correlation, 74 patients specimens were analysed using the original Triage BNP system and the BCA2. Deming regression analysis indicated a slope of 1.071 with a y-intercept of 8.8 ng/L. The correlation coefficient was 0.97. Since the observed BNP concentrations spanned the entire analytical range of 5–5000 ng/L, the data also confirmed linearity of the BCA2 method. The results of this study confirm acceptable analytical performance and indicate that the original Triage BNP system and Triage BNP on the Beckman Coulter Access 2 are interchangeable.

TP1.47

BENCE JONES PROTEIN ANALYSIS BY CAPILLARY ZONE ELECTROPHORESIS

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Aim: To develop a capillary zone electrophoresis (CZE) method for urine protein separation and Bence Jones Protein (BJP) analysis.
Twenty-nine urine samples were concentrated approximately 40-fold by centrifugal ultrafiltration (10 kDa molecular weight cut-off), and washed three times with 0.9% saline. Pressure was used to inject 19.31 nL of sample. Urine proteins were separated using a 0.05 mM di-sodium tetraborate buffer and a 30 kV separation voltage on the Beckman PACE/MDQ CZE platform, in a 21×25 μm fused silica capillary, at 25 °C, and detected by UV absorption at 214 nm. The electropherograms were interpreted by experienced Clinical Scientists, and compared to the original agarose gel electrophoresis interpretation. Samples were then checked for monoclonal light chains by immunofixation.
The CZE method was reproducible (the electroosmotic flow migration time CV was 0.12% ($n=10$)) and sensitive (0.09 g/L urine paraprotein detection limit). CZE analysis was more sensitive than agarose gel electrophoresis, detecting an additional immunofixation-confirmed BJP case. However, CZE was less specific than agarose gel electrophoresis. Eighty-three percent and 41% of samples underwent immunofixation based upon the CZE and agarose gel electrophoresis analyses, respectively. Using CZE interpretation rules reduced this figure to 72%.
Further rule and methodology refinement would improve the assay's specificity and practicability, and allow the development of immunosubtraction techniques for the identification of monoclonal light chains (replacing immunofixation). This would make the assay suitable for routine use and improve the quality of BJP analyses offered by the laboratory.

TP1.48

DEVELOPMENT AND VALIDATION OF AN APPLICATION FOR ANALYSIS OF METHOTREXATE ON THE HITACHI P MODULE CHEMISTRY ANALYSER

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Background: Patients with haematological malignancies who are candidates for treatment with high dose Methotrexate require rapid reporting of Methotrexate levels according to nationally agreed protocols. Locally, monitoring of levels at 24, 48 and 72 h is

required to determine the dosage of Folinic acid (Leucovorin) rescue needed to achieve a Methotrexate level of <0.10 μmol/L. Because of the practicalities involved in providing 24/7 service the assay would be best deployed on a routine chemistry analyser. In the absence of an analyser/method able to meet the required sensitivity, a unique application was developed.
Method: Employing a manual Enzyme Mediated Immunoassay Technique kit marketed by Dade Behring, an open channel method was developed and assessed on the Hitachi P Module. Sample type used was serum. Reaction conditions were optimized by varying buffer/reagent concentrations and assay timings. Two different mathematical models and 2 calibration spans were explored. Correlation studies were carried out against Abbott TDX (FPIA method) and assay performance characterised.
Results: Intra-run; target values 0.13 and 1.08 umol/L gave CVs of 3.4% and 2.7%, respectively. Inter-run; target values of 0.07 and 0.18 gave CVs of 19.1% and 11.1%, respectively. Precision profiling and functional sensitivity confirmed analytical range as 0.07–1.00 umol/L. Comparison with Abbott TDX gave Deming regression analysis slope of 1.0628 [95% CI: 0.9826–1.1430] intercept 0.0101 [95% CI: −0.0300–0.0502]. Pearson correlation $r=0.98$. On board reagent stability 12 weeks with 60–70 analyses realised per kit. Calibration interval 4–6 weeks. Unit cost-per-test approximately £14.00.
Conclusions: The new application provides a sensitive and robust analytical method to meet the clinical requirement.

TP1.49
CLINICAL UTILITY OF THE iQ®200 AUTOMATED URINE MICROSCOPY ANALYSER IN THE ASSESSMENT OF HAEMATURIA
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Aim: Microscopic analysis of erythrocyte morphology in urinary sediment is used to differentiate glomerular from non-glomerular sources of urinary bleeding. Bright-field microscopy (BFM) has limited precision, requires extensive manual labor and depends on the experience and skill of the observer. The iQ®200 Automated Urine Microscopy Analyser (iQ®200, Iris Diagnostics, USA), automates the microscopy by utilising Auto-Particle Recognition (APR™), a neural network trained to categorise 12 common urine components. We compared the iQ®200 with BFM for the determination of erythrocytes on strip positive urines.
Method: Each first morning urine was analysed within 4 h by:

1. Multistix 10 SGTM (Bayer, USA) and Clinitek 50TM (Ames Division, Miles laboratories Inc, Elkhart, Indiana, USA);
2. iQ®200;
3. BFM, the reference method, performed according to the European Urinalysis Guidelines.

Results: Erythrocytes were found in 328 of the samples examined. Forty-nine samples were dipstick negative for blood. Fifty-one samples showed <5 Erc/μL by the iQ®200, and negative by BFM. The remaining 228 samples were 1/+ to 3/+++ positive by dipstick. The iQ200 had sensitivity and specificity of 83% and 91%, respectively, in detecting non-glomerular bleeding among urine samples with positive dipstick for blood. There was good agreement between the methods (93%).
Conclusion: The iQ®200 was reliable when identifying non-glomerular haematuria. Although automated microscopy cannot provide all the detailed information obtainable by microscopic examination, its ability to identify the presence of low number of the erythrocytes (<5 Erc/μL) can be very important for the clinician. In our hands, the iQ®200 offers good recognition of erythrocytes as the basis for assessment of micro/macroscopic haematuria.

TP1.50
DETERMINATION OF MALONDIALDEHYDE IN BIOLOGICAL SAMPLES USING LIQUID CHROMATOGRAPHY WITH REFERENCE TO SAMPLE PREPARATION
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The most widely adopted method for measurement of oxidative stress is the estimation of the level of malondialdehyde (MDA), a stable product of the oxidative degradation of polyunsaturated fatty acids with more than two double bonds. This work refers to precise preparation of biological sample prior to HPLC analysis.
Our method is based on the formation of the complex between MDA and 2-thiobarbituric acid (TBA). We have tested the effect of chelating agent on lipoperoxidation in vitro during the derivatisation step, different solvents for extraction of the MDA-TBA complex, and recovery of method.
We found significantly increased levels of MDA in samples without addition of EDTA (1.11 ± 0.18) μmol/l, $n=69$ vs. (0.72 ± 0.13) μmol/l, $n=69$; Student's t test, $\mu=0.05$, $p<0.001$. Eight different solvents were tested: n-butanol, 1-propanol, 2-propanol, hexane, heptane, ethyl acetate, ethyl ether, and dichloromethane for extraction of the MDA-TBA complex. We found that only extraction with n-butanol led to complete recovery.
For construction of calibration curve we have prepared MDA standards by dilution aqueous solutions of malondialdehyde tetrabutylammonium in serum (1+9). The recovery of MDA was 97.3% (CV=3.8%).
This work proposes a highly specific and selective method for determination of MDA in biological samples.
This work was supported by grant MSM0021627502.

TP1.51
EVALUATION OF A NEW ENZYMATIC CREATININE ASSAY FOR KONELAB
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Creatinine is one of the high volume tests in clinical laboratories. The Jaffe method, even with numerous variations, is susceptible to interference from other sample constituents. Enzymatic creatinine assays are designed to eliminate the most common interferences in Jaffe techniques.

In this study we have evaluated the performance of a new Konelab creatinine assay (Thermo Electron Corp, Vantaa, Finland). This method is based on enzymatic degradation of creatinine followed by reaction with creatininase, creatinase and sarcosine oxidase. The liberated hydrogen peroxidase reacts with 3-hydroxy-2,4,6-triiodo benzoic acid and 4-aminoantipyrine to form a phenol dye with absorbance at 545 nm. Konelab uses a two reagents method with end-point measurement at 540 and 700 nm.

The performance characteristics of the assay were analyzed using Thermo Electron's Konelab 30 analyzer. In comparison studies the results measured with new Konelab kit were compared with those analyzed with Roche Creatinine Plus kit (Roche Diagnostics GmbH, Mannheim, Germany).

Within run imprecision (CV%, $n=20$) was 4.75%, 3.16% and 1.52% at the level of 46, 101 and 735 μmol/l, respectively. The detection limit was 6.2 μmol/l and measurement range up to 2000 μmol/l. Correlation coefficient between Konelab and Roche methods was 0.995 ($n=50$) and Passing/Bablok regression $y=1.028x-0.083$.

There is no significant interference when ascorbic acid concentration is below 20 mg/l, triglycerides below 11 mmol/l and bilirubin below 340 μmol/l.

The new Konelab creatinine test eliminates sample interferences. This study demonstrates that the evaluated method is precise and correlation with the other enzymatic methods is excellent. It is a cost-effective and reliable assay for small and large scale use, especially in automated laboratories.

TP1.52

DETERMINATION OF BOTH REDUCED AND OXIDISED GLUTATHIONE USING LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION

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A method for measurement of both reduced (GSH) and oxidised (GSSG) glutathione in plasma, erythrocytes and hepatocytes has been developed and evaluated.

Reduced glutathione reacts with orthophthaldehyde (OPA) to form a stable, highly fluorescent tricyclic derivative at pH 8, while GSSG reacts with OPA at pH 12. At measurement of GSSG, GSH was complexed to *N*-ethylmaleimide.

For the separation, reverse phase column Discovery C18, 150 × 4 mm, 5 μm, was used. The mixture of methanol and 25 mmol/l phosphate buffer (15:85, v/v), pH 6.00, was used as mobile phase. Excellent linearity was achieved. The intra- and inter-assay coefficients of variation were 3.8% and 5.2%, respectively. The recovery was 95–105%, and limit of detection was 0.1 pmol of glutathione adduct.

We have developed a highly selective, sensitive and rapid method for measurement of both GSH and GSSG in biological samples.

This work was supported by grant MSM0021627502.

TP1.53

CARBAMAZEPINE MEASUREMENT BY KIMS IMMUNOASSAY: REPRODUCTIBILITY AND CORRELATION ASSESSMENT

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Objective: To validate an assay based on kinetic interaction of micro-particle in solution (KIMS) technology implemented in a MODULAR P (Roche Diagnostics).

Methods: Three levels of Roche ONLINE TDM controls were used to evaluate imprecision. Within-run (WR) imprecision was performed running each control level for 3 days ($n=60$). Between-run (BR) imprecision was assessed processing each control level for 20 days. Inaccuracy was carried out using 95 patient's serum samples from TDM routine analysis stored at −20 °C. We compared KIMS assay with FPIA (AxSym-Abbot Diagnostics) and dry chemistry (Vitros950-Johnson and Johnson CD).

Limit of detection was calculated running serum free of carbamazepine ($n=20$).

Results: Carbamazepine mean values were 2.4, 7.2 and 15.6 μg/ml for each control level. WR CVs were 4.04%, 3.89%, and 2.34%, and BR CVs were 6.28%, 4.77%, and 5.75% for the three control levels studied.

We were not able to obtain detection limit because protocol data were consistently zero. In the comparative studies the correlation coefficients were 0.995 versus Vitros950 assay and 0.991 versus AxSym. Deming regression analysis yielded a slope 1.055 (95% C.I.: 0.875–1.342) and an intercept of 0.060 (95% C.I.: −0.025–0.142) using Vitros950 as reference method, and a slope 1.055 (95% C.I.: 0.875–1.342) and an intercept of 0.060 (95% C.I.: −0.025–0.142) when AxSym was the reference method. Passing–Bablok agreement test yielded similar results.

Conclusions: Imprecision results met the analytic goals proposed by NACB. Statistical tests of comparative analysis did not indicate differences regarding the two reference methods.

TP1.54

DETERMINATION OF ASCORBIC ACID IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION WITH A VIEW TO STABILITY

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A method for the measurement of ascorbic acid (AA) using high-performance liquid chromatography with ultraviolet detection and investigation into the protein precipitation techniques with regard to stability and recovery are described.

The aim of this work was to establish the most suitable precipitant reagent for the determination of AA with respect to stability and recovery.
The effectiveness of various protein precipitants was tested as follows. To plasma and AA solution (50.0 μM), the following different precipitant reagents were added: meta phosphoric acid (5%), perchloric acid (1.0 M), trichloroacetic acid (10%), sulfosalicylic acid (10%), acetonitrile with hydrochloric acid, acetonitrile with acetic acid, methanol with hydrochloric acid, and ethanol with hydrochloric acid. The only two protein precipitants that led to satisfactory recoveries of AA were metaphosphoric and perchloric acid. Stability of AA samples for analysis was investigated over 10 h.
Ascorbic acid samples extracted by metaphosphoric acid were stable on a cooled autosampler (4 °C) for at least 10 h (with a decline 1.8% for AA solution and 2.8% for plasma). Perchloric acid as protein precipitant for AA was unsuitable (36.0% for AA solution and 7.3% for plasma).
Analytical performance of this method is satisfactory. The intra- and inter-assay coefficients of variation were 2.1% and 5.8%, respectively. The calibration curve was linear with the tested range 2.0–250.0 μM. The recovery was 96.1% (CV=4.8%) and the limit of detection was 25 pmol.
This assay is a highly sensitive and reproducible HPLC method for the determination of ascorbic acid in human plasma.
This work was supported by grant MSM0021627502.

TP1.55
QUANTITATIVE DETERMINATION OF HUMAN SERUM GELSOLIN: DEVELOPMENT AND VALIDATION OF AN AUTOMATED TURBIDIMETRIC IMMUNOASSAY
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Gelsolin has been shown to sever assembled actin filaments in two. Upon cell death and tissue injury actin is released into the circulation. Actin filament formation in the circulation may lead to microthrombi formation, endothelial injury and multi organ failure. The plasma gelsolin concentration is inversely correlated to the released actin and can predict the risk of developing fatal respiratory failure after allogeneic stem cell transplantation or major trauma. In the present study an automated turbidimetric immunoassay for determination of human plasma gelsolin was developed and validated.
Immune complexes were formed, by mixing rabbit anti human gelsolin and antigen. The resulting changes in transmitted light, was quantified by a Cobas Mira Plus automatic analyser. The optimised measuring range was 8–266 U/L. Calibration was performed using a 6 point calibration curve. Calibrator material was a serum pool obtained from healthy donors, value assigned to 100 U/L.
The estimated within and between run and total imprecision were below 2.3%, 5.8% and 6.2%, respectively. Analysis of serial dilutions of serum in the range 13–121 U/L showed good linearity (less than 8% deviation from the expected value) in the assay. Detection limit was estimated to 1 U/L. No significant interference (less than 10% deviation from initial value) was seen after addition of 600 mg/L bilirubin, 2.5 g/L triglyceride or 1.5 g/L haemoglobin to serum. The measured security range was 266–970 U/L.
This assay represents an automated, fast and precise alternative to other immunoassays for determination of human gelsolin in serum and may prove a valuable tool in the early identification of patients at risk of developing multi organ failure.

TP1.56
A NON-ISOTOPIC ASSAY FOR HOLO-TRANSCOBALAMIN
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Cobalamin (vitamin B12) is important in many body processes including synthesis of DNA, red blood-cells and the nerve cell myelin-sheath. Deficiency may lead to potentially irreversible neuro-psychiatric disorders and megaloblastic anaemia; vitamin B12 deficiency is believed to be under-diagnosed, especially in elderly people. At present, identification of those requiring therapy is hampered by the poor diagnostic accuracy of conventional tests. It has been proposed that holo-transcobalamin (holoTC) may provide a better index of functional cobalamin status, and a possible early marker of vitamin B12 deficiency. A competitive radio-immunoassay (RIA) has been commercialised providing a sensitive and reliable method for the quantitative determination of holoTC in serum and plasma. However, the RIA requires a large sample volume (0.4 ml) and is difficult to automate. A requirement has been recognized to develop an automatic non-isotopic, direct assay using a smaller sample volume.
We have developed a microparticle enzyme-immunoassay (MEIA) for holoTC that utilizes 2 well-characterised binders that can immobilise and detect holoTC specifically. In this MEIA, performed on an Abbott AxSym® analyser, a bead-bound monoclonal antibody specific for holoTC immobilises the holoTC from the sample, subsequently the immobilised holoTC is quantified using a second monoclonal antibody specific for TC. This direct MEIA has an analytical range from <10 pM to over 200 pM holoTC and typically has an imprecision of less than 5%. The assay shows equivalence with the RIA, with a 20 min "time to first result" and a throughput of 50 samples/per hour.

TP1.57
ANALYTICAL CHALLENGES IN THE MEASUREMENT OF IGF-1: DISPLACEMENT VERSUS EXTRACTION
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Insulin-like growth factor 1 measurement is used in the diagnosis of acromegaly and investigation of short stature. In serum IGF-1 circulates as a ternary complex consisting of a binding protein and an acid labile subunit. Prior to immunoassay, IGF-1 must be displaced from this complex and reassociation prevented. This can be accomplished by acid-ethanol extraction (in-house RIA) or acid treatment and blocking e.g. by IGF2 (DPC IMMULITE 2000, Nichols Advantage).

These methods were compared using serum from normal individuals and sub-population groups in which proteolysis of binding proteins might affect the displacement process e.g. pregnancy.
The results showed that both methods compared well with the RIA method except for samples from pregnant women. Regression analysis using Excel showed R^2 values for the IMMULITE 2000 and Nichols Advantage comparison with the RIA of 0.98 and 0.93, respectively (regression equations $y=1.0996x-4.1675$ and $y=0.9384x-0.6917$), in 120 normal individuals, but in pregnant subjects the regression equations were $y=0.8429x+1.4145$ and $y=1.0265x-4.1087$.
In pregnancy, proteases that modulate IGF binding proteins are increased. The results could reflect the effect of proteolysed binding proteins.
The displacement assays compared well with acid/ethanol RIA but displacement efficiency may be compromised under different pathophysiological conditions. The accuracy of non-extraction IGF-1 assays must be established where changes in binding proteins can be anticipated.

TP1.58

IR-EXCITATION ANTI-STOKES PLATE FLUOROMETER FOR UP-CONVERTING PHOSPHOR-BASED ASSAYS

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Up-converting (UPC) inorganic lanthanide phosphors generate intense anti-Stokes photoluminescence under infrared (IR) illumination; Y2O2S host lattice doped with Er3+ and Yb3+ produces narrow emission bands at 520–550 nm and 650–670 nm. The emission bands are at shorter wavelengths than excitation light and thus totally discriminated from autofluorescence and scattered excitation light even without temporal resolution. The major potential advantage of the UPC phosphors as a label in an immunoassay is the excellent detectability with an uncomplicated detection system.
Commercial fluorescence plate reader with epifluorescence setup was modified for anti-Stokes photoluminescence intensity measurement by replacing Xenon flash tube with a 980 nm laser diode with output power of 200 mW. The excitation pathway was converted to reflect the focused laser beam directly to a microtitration well using a small diameter aluminium mirror. The excitation filter was a long-pass filter glass RG-850. Photoluminescence emission was collected to a band-pass emission filter mounted in the emission pathway and focused on the photocathode of the photomultiplier tube to be counted simultaneously with laser excitation. The anti-Stokes photoluminescence was measurable with background count rate equivalent to that achieved in luminescence measurement (less than 50 cps). Dynamic range of the measurement was over five orders of magnitude. The instrument was able to measure 256 non-overlapping anti-Stokes photoluminescence data-points with spatial resolution less than 500 μm from a single well enabling an array-in-well approach.
The unique photoluminescence properties of the up-converting phosphors enabling low limit of detection and the inexpensive IR laser diode-based measurement configuration render the up-conversion an attractive alternative to the ultraviolet-excited time-resolved fluorescence of lanthanide chelates.

TP1.59

A COMPETITIVE TIME-RESOLVED FLUOROIMMUNOASSAY FOR THE SCREENING OF MONENSIN RESIDUES IN EGGS

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Coccidiostats are mainly used as feed additives in the poultry industry to prevent and treat a parasitic infection called coccidiosis. Polyether ionophores, which are perhaps the most widely utilised group of coccidiostats, may cause adverse health effects on humans by influencing the cardiovascular system. There is evidence that coccidiostat residues can be found in poultry tissues and eggs. Consequently, rapid methods, which can be used to screen foodstuffs for these harmful residues, are needed. The statutory residue control in the EU is defined in Directive 96/23/EC.
We developed a rapid screening method, which could be utilised in a quantitative or qualitative mode, for ionophore monensin residues in eggs. Prior to the time-resolved fluoroimmunoassay, the residues were extracted from eggs with acetonitrile according to a simple protocol. The method validation was performed in compliance with Commission Decision 2002/657/EC. The CCβ-value (detection capability) of the assay was determined to be less than 2 ng/g. The mean recoveries, determined at three concentration levels ($n=6$), varied from 88.0 to 102.1%. The intra-assay variations ($n=6$) were typically less than 10% and interassay variations ($n=18$) ranged between 10.3 and 13.9%.
The described method is user-friendly; the extraction protocol is simple and the results from the immunoassay are available in less than an hour. Therefore, the method may prove to be applicable to routine analysis.

TP1.60

IS A 24 H URINE COLLECTION NECESSARY FOR SCREENING FOR PHAEOCHROMCYTOMA?

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It is well known that samples presented as 24 h urine collections do not, in many cases, represent an accurately timed 24 h collection. In order to evaluate this we looked at 540 24 h urine samples collected for Homovanillic acid (HMMA) determination in the department over a three month period in late 2004.
Samples were analysed for HMMA and urine creatinine concentration. 24 h HMMA and HMMA creatinine ratios were determined. The mean ($\pm$1 S.D.):for HMMA was 22.7($\pm$17.3) umol/24 h and HMMA creatinine ratio 4.0($\pm$4.0) μmol/mmol creatinine. Of these 540 samples 370 were within our reference range (HMMA <30 μmol/24 h: HMMA:creatinine (<4.7 μmol/mmol creatinine)) by both methods. 42 samples were abnormal by both methods. There were discordant results between the remaining 128 samples. In 100 samples the 24 h urine HMMA was normal with a raised ratio and in 28 samples there was an abnormal HMMA but a normal HMMA:creatinine ratio. In the discordant results we identified 28

samples with urine volumes >3.00 L and 9 with volumes <600 mL. Furthermore in the 28 discordant high 24 h VMA samples 13 samples had high 24 h urine creatinine (>17.7 mmol/24 h).
We have demonstrated in our series that in a significant number of samples the 24 h urine collections may not have been an accurate collection. Our data suggest that the HMMA:creatinine ratio may be a better reflection of HMMA excretion. These results suggest that 24 h urine collections may not be necessary for HMMA determination. Further work needs to be carried out to confirm these findings.

TP1.61

COMPARISON OF TWO AUTOMATIC BIOCHEMICAL ANALYSERS: ADVIA 1200 AND HITACHI 912 USED IN THE ROUTINE WORK OF CLINICAL LABORATORY

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Introduction: The ADVIA 1200 Chemistry System is an automated, clinical chemistry analyser performing 800 photometric tests per hour, which similarly to the HITACHI 912 allows operators to view calibration curves to check the calibration results and verify daily precision control before their routine analyses. Analysers also have statistics menu for calculation of standard deviation and coefficient of variation for precision. The aim of the study was to compare two biochemical analysers: ADVIA 1200 and HITACHI 912, used for assessment of substrate analysis. We evaluated the analytical precision of determinations, analytical accuracy and compared the results obtained from both analytical systems.
Material: Examinations were performed on 180 sera from cancer patients in different stages of disease and course of treatment, 50 from cardiology and 24 from patients of internal medicine department.
Results: Statistical quality control showed a good precision and accuracy of all assessed determination methods. Student *t*-test showed significant differences between the results from both analysers, and coefficients of correlation were above 0.985, apart from calcium, magnesium, total bilirubin and LDL cholesterol which ranged from 0.94 to 0.965. To assess the agreement between the results from two analysers Bland and Altman graph was used as they allow to look for any systematic bias and to identify possible outliers. We obtained higher differences between the results for high urea and uric acid concentrations. The test for linearity showed lack of significant deviation from linearity for all methods.
Conclusion: ADVIA 1200 and HITACHI 912 are good quality analysers, and fully comparable systems, but ADVIA 1200 is a considerably quicker instrument.

TP1.62

STABILITY OF PARATHYROID HORMONE IN BLOOD FROM HAEMODIALYSIS PATIENTS: ROCHE ELECSYS (E170) METHOD

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Parathyroid hormone (PTH) is measured in patients with chronic renal failure, as an indicator of bone turnover. PTH is known to be rapidly metabolised in vivo, and has been shown to be unstable when stored at room temperature. This may be of concern when samples are collected at sites distant from the laboratory, or outside normal operating hours. Recent studies have suggested that PTH is more stable in blood collected into potassium-EDTA tubes, and several manufacturers now recommend this as the sample of choice. The aim of the study was to compare PTH stability in serum and EDTA-plasma from blood collected from 17 pre-haemodialysis patients. Samples were allowed to stand for 2, 4, 6 and 24 h at room temperature, and PTH was determined by the Elecsys PTH assay on the Roche E170 automated immunoassay analyser. Ethical approval for the study was obtained, and all patients gave informed consent. Data were analysed using the Wilcoxon paired-signed ranks test. At baseline, PTH in serum was 26% higher than in plasma. When PTH levels at each time point were compared with the zero-time point values, PTH in serum was significantly reduced at 6 and 24 h ($p=0.016$ and $p=0.0002$, respectively). There was no significant change in the level of PTH in EDTA-plasma over 24 h.
Results indicate PTH to be more stable in plasma-EDTA than serum when stored at room temperature for up to 24 h, using the Roche Elecsys (E170) method. However, the discrepancy between baseline serum and plasma PTH is of concern; possible mechanisms for this difference are discussed.

TP1.63

PURINE BIOSYNTHESIS RIBOSIDES – SYNTHESIS, SPECTRAL PROPERTIES AND HPLC ANALYSIS

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Only three inherited metabolic defects are known in the ten-step pathway of purine de novo synthesis (PDNS). The intermediates of the second part of the pathway (from aminoimidazole ribotide to IMP) have closed aminoimidazole ring and so are expected to be detectable by separation techniques via UV absorptivity. We expect that analogous ribosides can be probably detected in biofluids from patients with potential defects of the purine de novo synthesis.
We have synthesized and purified the ribosides of interest. HPLC assays were performed using Waters Spherisorb ODS 2 (5 μm, 4.6 × 250 mm) column protected by MetaGuard 4.6 mm Polaris (5 μm, C18) with diode array detection. All the compounds of interest were separated using linear gradient of buffer A (40 mmol/l ammonium acetate, pH 5) to buffer B (30% acetonitrile: 70% buffer A) over 36 min.
The ribosides were baseline separated under the given conditions. All compounds provide UV spectra with molar absorption coefficients more than 6000 in the range of 240–270 nm. Potential usefulness of the method was demonstrated on samples from patients with known deficiencies, urines from healthy persons with added compounds and Chinese hamster ovary cells defective in the steps of purine de novo synthesis.

HPLC is useful and effective tool for analysis of aminoimidazole ribosides which enables diagnosis of known as well as not so far identified inherited defects of PDNS pathway.
This work was supported by grant IGA NR/7796-3 and NR/8578-3.

TP1.64

THYROID FUNCTION TESTS AND TUMOR MARKERS: COMPARISON BETWEEN MODULAR E170 AND AUTODELFIA IMMUNOASSAY SYSTEMS AND MATRIX CHANGE FROM SERUM TO PLASMA

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We have evaluated the analytical performance of the thyroid function tests (TSH, FT4 and FT3) and two tumour markers (total PSA, free PSA and CEA) on a Modular E170 analyser (Roche Diagnostics).
Lithium heparin plasma samples measured with Modular E170 were compared with serum samples analysed with AutoDelfia (Perkin-Elmer). In all methods the overall correlation in routine samples was good, however, systematic biases between methods were observed. The TSH and FT4 results on Modular E170 were on average 23% and 12.5% higher, whereas FT3 results were 24% lower than on AutoDelfia. Correspondingly, total PSA, free PSA and CEA results were 6%, 0.4% and 12% higher on Modular E170. When measurements were done with serum samples in both analysers similar differences between methods were observed. Since no difference between serum and plasma samples was observed both specimen types can be used on Modular E170 assays. Day-to-day variations for thyroid assays and tumour markers were less than 5% and 3% for all three control levels tested.
In order to shorten turnaround time and efficient use of reagents and technician workload the reflex-test option on Modular E170 was adapted in use. Accordingly, we were able to selectively run free PSA and FT4 assays on samples with predefined levels of total PSA and TSH, respectively, obviating the need to manually select samples for complementary assay batches. The reflex-test option has also been used for automatic selection for dilution tests of 22 TSH samples with discrepant TSH and FT4 results. None of these samples were found to have analytical interference in the TSH assay.

TP1.65

COMPARISON OF TWO ASSAYS FOR CA125 MEASUREMENT (ARCHITECT vs. AxSYM)

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Introduction: Monitoring of ovarian cancer patients for response to therapy and for recurrence of disease is aided by CA 125 measurements. Recently, a new chemiluminiscent microparticle assay has been developed for the measurement of CA 125 in ARCHITECT instrument system (Abbot Laboratories).
Objective: To compare ARCHITECT CA 125 assay and AxSYM CA 125 assay (MEIA), before used in our laboratory.
Method: Serum CA 125 level was simultaneously measured in 50 serum samples in ARCHITECT i2000 and AxSYM analyzers. Comparison of methods was assessed by regression analysis (Passing-Bablok regression) and Intraclass Correlation Coefficient (ICC).
Results: In this evaluation serum CA125 levels ranged from 3.2 to1526 U/mL in ARCHITECT i2000 analyzer and between 2.55 and 1476 U/mL in AxSYM instrument system.
Regression equation: [ARCHITECT CA 125 assay]=0.045+ 1.340*[AxSYM CA 125 assay]
Constant=0.045 (IC 95%=−0,598 − 0.967)
Slope=1.340 (IC 95%=1.276 − 1.377)
ICC=0.989 (IC 95%=0.973 − 0.995).
Conclusions: Although ICC shown a good agreement between both methods, proportional bias demonstrated in Passing-Bablok regression does not allow the direct interchange values between compared methods.

TP1.66

HOMOGENEOUS IMMUNOASSAY FOR THYROID-STIMULATING HORMONE BASED ON FLUORESCENCE RESONANCE ENERGY TRANSFER

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Thyroid-stimulating hormone (TSH) stimulates the thyroid gland to release two hormones: triiodothyronine (T3) and thyroxine (T4), which control metabolism of all cells. By measuring the amount of TSH in blood the activity of the thyroid gland can be determined. Normal levels of TSH in adults are usually from 0.4 to 6.7 mIU/L, but may vary significantly in disease. Therefore TSH assays should have good sensitivity and have a wide dynamic range. The aim of our study was to develop a novel homogeneous immunoassay capable of measuring TSH levels in serum samples.
The developed immunoassay for TSH was based on fluorescence resonance energy transfer between a donor and an acceptor. As a donor we used a long-lifetime europium (III)-chelate conjugated with a monoclonal antibody and as an acceptor a short-lifetime near infra-red fluorescent molecule conjugated with a recombinant fragment of an antibody. When the donor and the acceptor were both bound to TSH, they were close enough to participate to the energy transfer generating sensitised emission, which could be measured with time-resolved detection. Background originating from autofluorescence of biological material was minimised with time-resolved detection, thus improving the sensitivity of the assay.
We were able to develop a separation free homogeneous immunoassay for TSH, which was easy to automate and fast to perform compared to assays requiring washes. The optimised assay had the lowest limit of detection approximately 8 mIU/L and the dynamic range was over two decades. Thus the assay was sensitive enough to detect TSH levels of patients with hypothyroidism.

TP1.67

AN IMPROVED URIC ACID ASSAY FOR USE ON THE OLYMPUS AU400™, AU640™ AND AU2700™ ANALYSERS

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Aims: To evaluate the application of a new Olympus Uric Acid (OSR6198) assay with improved interference performance on the Olympus AU400, AU640 and AU2700 analysers.
Methods: Uric acid is converted by uricase to allantoin and hydrogen peroxide. The formed H2O2 reacts with *N*,*N*-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt and 4-aminophenazone in the presence of peroxidase to produce a chromophore, which is read biochromatically at 660/800 nm. The amount of dye formed is proportional to the uric acid concentration in the sample. The assay is calibrated using a single point multicalibrator (66300) traceable to Nist 909b. The assay can be used for the measurement of serum/plasma or urine.
Results: Lipemic, haemolytic, icteric and ascorbate interference are all <5% (current Olympus assay <20%) at levels (1000 mg/dl Intralipid, 500 mg/dl haemoglobin, 40 mg/dl unconjugated bilirubin and 20 mg/dl ascorbate) using NCCLS EP7-P.
The reagent has a 30 day on board and calibration stability and is linear within a range of 90–1700 μmol/l for serum and 120–23,000 μmol/l for urine.
Imprecision CV values on the AU2700 for serum (range: 182–1380 μmol/l) and urine (range: 1250–5600 μmol/l) are <1% serum and <1.5% urine for within run precision and <1.5% serum and <3% urine for total CV using NCCLS EP5-T2.
Linear regression carried out according to NCCL EP9-A against the existing Olympus assay (*X*) showed good regression.
Serum: [Y(OSR6198)=1.015X−2.16; r=0.999; n=120, range:108–1581 μmol/l].
Urine: [Y(OSR6198)=1.024X+121; r=0.994; n=102, range:337–7667μmol/l].
Conclusions: The assay (OSR6198) shows excellent performance characteristics and much improved interference performance over the current Olympus Uric Acid assay and is suitable for use on the Olympus chemistry analysers.

TP1.68

EVALUATION OF THE TRITURUS ENZYME IMMUNOASSAY AUTOANALYZER FOR THE MEASUREMENT OF b2-GLYCOPROTEIN I

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Introduction: Triturus is an open, flexible, completely mechanized, multianalysis, multiseries analyser, with possibility of online connection. Aim of the study: The objective of this study is to evaluate the reliability of the Triturus Enzyme Immunoassay Autoanalyzer of GRIFOLS for the measurement of b2-Glycoprotein I according to IFCC criteria, NCCLS and ECCLS Guidelines. Methods: Protocol: We have studied intra and inter-assay replications employing 30 samples of each level of Control (Positive and Negative Internal Control of the Kit) in one same run and one sample day in of each control over the next month. As well as the detection limit measuring of this parameter in 5 samples of distilled water. For the study of linearity several dilutions (6 levels of concentration) were performed. Statistical Method: Statistical analysis of data was performed using Statistical Package SPSS (Version 12.05). Results: Precision: Intra-assay: Coefficient of Variation (CV) (CV1, CV2) Inter-assay: (CV1, CV2) and Detection Limit (Ld). b2-Glycoprotein I Ig M: Intra-assay: CV1=0.l82, CV2=0.66; Inter-assay: CV1=4.50, CV2=2.80 b2-Glycoprotein I Ig G: Intra-assay: CV1=1.43, CV2=1.03; Inter-assay: CV1=4.30, CV2=2.10 Ld=Xb+K×sb=0.0364+2.5390×0.0010=0.0389 Linearity: Correlation between observed(o) and expected(e) values. b2-GLYCOPROTEIN I IG M o=−0.748+0.988b2-GLYCOPROTEINIIG M e r^2=1 p<0.0001 b2-GLYCOPROTEINI IG G o=−0.458+0.944b2-GLYCOPROTEINI IG Ge r^2=1 p<0.0001 Conclusions: 1—The study of Precision, Linearity and Detection Limit guarantee analytical quality. 2—The analytical CV of all parameters studied amply observe the appropriate decision criteria of the Quality Commission of IFCC.

TP1.69

THE BECKMAN ACCESS® PROLACTIN ASSAY; PREVALENCE AND DETECTION OF HYPERPROLACTINAEMIA DUE TO MACROPROLACTIN

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Hyperprolactinaemia due to macroprolactin (MPRL) can lead to misdiagnosis and inappropriate treatment and laboratory screening of all samples with elevated total prolactin (PRL) to detect MPRL has been advocated.
We investigated the reactivity of the Access PRL assay with MPRL and a method for the detection of MPRL in samples characterised by the Wallac DELFIA PRL assay with PEG precipitation and gel filtration chromatography.
In 36 samples containing MPRL (total PRL (DELFIA) 739–4850 mIU/L, monomeric PRL 111–506 mIU/L) the Access assay gave lower results, range 227–864 mIU/L. In 192 samples with total PRL >700 mIU/L, from 6 laboratories using the Access assay, total PRL by DELFIA was 423–9230 mIU/L and MPRL was identified as the cause in 4 cases. Because PEG interferes in the Access assay we investigated measurement of PRL in the precipitate after re-dissolution as a test for the presence of MPRL. With the DELFIA assay and a serum pool containing no significant MPRL we recovered a mean 19% of PRL in the precipitate, CV 18.5%. In a pool with 78% MPRL mean recovery of PRL was 76%, CV 11.4%. Using the Access assay with 18 samples containing no significant MPRL and total PRL 728–3686 mIU/L, mean recovery of PRL in the PEG precipitate was 20%,

range 10–36%. In 2 samples containing MPRL, recovery was 58 and 60%.
We conclude that the prevalence of hyperprolactinaemia due to MPRL with the Beckman Access PRL assay is relatively low and approximately 2% of all cases with elevated total PRL. The PEG precipitation test, with measurement of PRL in the precipitate is a promising approach to the detection of MPRL.

TP1.70

NT-PROBNP ON DIMENSION®-HM (DADE BEHRING): EVALUATION OF THE ANALYTICAL PERFORMANCE CHARACTERISTICS AND CORRELATION WITH ROCHE ELECSYS ASSAY

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Plasma B-type natriuretic peptide (BNP) and N terminal proBNP (NT-proBNP) are promising markers for heart failure diagnosis, prognosis and treatment. Their elevated baseline levels have been associated with adverse long-term outcome in patients with acute coronary syndromes.
Recently Dade Behring marketed an automated NT-proBNP assay (PBNP) on Dimension®-HM system. We evaluated the analytical performance characteristics and agreement with Roche Diagnostics NT-proBNP results obtained using Elecsys instrument according to "Valtec" protocol.
The PBNP method is a one step enzyme immunoassay based on the sandwich principle. Sample is incubated with chromium dioxide particles coated with polyclonal antibodies and a conjugate reagent (alkaline phosphatase ALP). After separation and washing, the particle/NT-proBNP/conjugate sandwich is transferred to the cuvette where the sandwich bound ALP triggers an amplification cascade.
Total imprecision was <9% CV at NT-proBNP concentrations of 100–10,000 pg/ml on quality controls and pooled patients' plasma. Analytical sensitivity was found to be 9.8 pg/ml after the calibration and 10.1 pg/ml 10 days after. Dilution linearity/recovery evaluated with a NT-proBNP concentration >25,000 pg/ml by dilution and overall recovery was between 98.4 and 104.7%. No hook effect was observed at NT-proBNP 96,000 pg/ml concentration. Triglyceride was not an interfering substance (up to 7 mmol/L). Hemoglobin interfered negatively with assay upper 240 μmol/L but less than 10% at 450 pg/ml. A precision profile demonstrated a total imprecision of 10% CV at a NT-proBNP concentration of 70 pg/ml and 20% at 30 pg/ml. Dimension® results highly correlated ($r=0.99$) with Roche Elecsys NT-proBNP.
The NT-proBNP Dade Behring assay demonstrated excellent analytical performance characteristics and agreement with NT-proBNP results on Elecsys instrument.

TP1.71

EVALUATION OF NEW OLYMPUS URINE CALIBRATOR (ODC0025)

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Aims: Compare the quantitative determination of amylase, calcium, creatinine, glucose, inorganic phosphate, magnesium, urea and uric acid in urine using the new Olympus urine system and the previous recommended Olympus urine system.
Method: Method comparisons were performed in accordance with NCCLS EP9-A for each parameter using: (1) new Olympus urine system: Olympus urine calibrator (ODC0025) and CE marked settings. (2) old Olympus urine system: Olympus system calibrator (OE66300) and non-CE marked settings.
Results: Method comparison between Olympus old and new urine systems delivered the following regression equations and biases where significant patient shift is expected: amylase OSR6106: $Y=1.067X-4.492$. bias=+6%, amylase OSR6182: $Y=1.159X+0.543$. bias=+16%, calcium OSR6113: $Y=0.971\times0.004$, calcium OSR6176: $Y=0.982X-0.016$, creatinine OSR6178: $Y=0.812X+73.733$. bias=+15% , glucose OSR6121: $Y=1.001X-0.008$, inorganic phosphorous OSR6122: $Y=0.938X+0.122$. bias=−6% ,magnesium OSR6189: $Y=0.938X+0.122$, urea OSR6134: $Y=1.144X-3.347$. bias=+13%, uric acid OSR6136: $Y=1.029X-11.092$.
Conclusion: Calcium (OSR6113 and OSR6176), glucose OSR6121, magnesium OSR6189 and uric acid OSR6136 show equivalent performance between old and new Olympus urine systems. Amylase (OSR6106 and OSR6182), creatinine OSR6178 and urea OSR6134 gave positive biases whilst inorganic phosphorous OSR6122 gave a negative bias. The expected patient shift and superior recovery of external quality proficiency samples are due to improved matrix properties and traceability for the new Olympus urine calibrator. The set points are traceable to internal Reference Standards or methods of higher order. The new Olympus urine system complies with IVDD requirements.

TP1.72

COMPARISON TEST OF STREPTAVIDIN-COATED MICROTITRATION PLATES

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Streptavidin (SAv)-coated microtitration plates provide a straightforward means for immobilisation or separation of any biotinylated molecules for in vitro-diagnostic and research purposes. SAv-coated 96-well plates from commercial suppliers (six plates) and our laboratory (two plates) were tested with respect to their binding capacity for small and large molecules, leaching, well-to-well variation within a plate and immunoassay performance.
The binding capacities for europium-labeled biotin (Eu-biotin) varied, depending on the plate, from 4.4 to more than 150 pmol/well and for biotinylated monoclonal antibody (IgG) from 1.2 to 6.4 pmol/well (190–1030 ng). One hour's incubation in assay buffer resulted in leaching of 0.5 to 76 ng/well of SAv from non-prewashed wells and 0.5 to 60 ng from prewashed wells. The desorbed quantity represented 0.002%–3.37% of the plates' total binding capacities. The well-to-well variations (CV%-values) within

a plate were from 1.2% to 8.0% when tested with respect to the maximum binding capacity.
Sandwich-type immunoassays were run for thyrotropin (TSH) using time-resolved fluorometric measurement of Eu-chelates directly from the surface, as well as for cancer antigen 125 (CA125) and prostate-specific antigen (PSA) utilising ELISA-tests from CanAg Diagnostic AB (Gothenburg, Sweden). The signal to background ratios, variation of the replicates and non-specific-binding characteristics were considered when evaluating the plate performance in the immunoassays. It was found that good performance relative to capacity, homogeneity or leaching did not necessarily lead to good overall assay performance. Rather, unwanted properties like non-specific binding and variation tended to increase in the plates with the highest capacities, whereas some low or medium capacity plates fulfilled the assay requirements optimally.

TP1.73

THE SIMULTANEOUS DETERMINATION OF UBIQUINOL-10 AND UBIQUINONE-10 IN HUMAN PLASMA USING HPLC WITH ELECTROCHEMICAL DETECTION

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Coenzyme Q10 is an important endogenous lipid-soluble antioxidant. Ubiquinol-10, the reduced form of coenzyme Q10 protects cells from oxidative damage. The oxidised form of coenzyme Q10 is known as ubiqinone-10. The ubiquinol-10/ ubiquinone-10 ratio may be used as a marker for oxidative damage.
The aim was to develop a HPLC method for the simultaneous determination of ubiquinol-10 and ubiquinone-10 in human plasma.
Plasma for analysis was collected into vacutainer tubes containing EDTA. Tubes were centrifuged and extracted with hexane and methanol. Sample was injected onto 125 × 4 mm RP-C18 column (Purospher, 5 μm; Merck). The mobile phase consisted of methanol-2-propanol-sodium acetate. We filtered this mobile phase through a 0.45 μm nylon filter and than degassed before use. The flow rate was 1 ml/min and temperature was set to 37 °C.
We detected coenzyme Q10 with ESA Coulochem II 5100A electrochemical detector. After separation, the components passed the conditioning cell (−600 mV) and ubiquinone-10 was reduced to ubiquinol-10. We detected ubiquinol-10 using Model 5010 analytical cell and with electrode 1 set at screening potential of −150 mV and electrode 2 set at +600 mV. We used ubiquinone-9 as internal standard.
Analytical performance of this method is satisfactory, both intra-assay and inter-assay were with coefficient of variation below 10%.
We have developed a HPLC method for the simultaneous determination of ubiquinol-10 and ubiquinone-10, which showed satisfactory analytical sensitivity, precision and linearity. This method is suitable for the measurement of both forms coenzyme Q10 in biological samples.
This work was supported by grant MSM 0021627502.

TP1.74

EVALUATION OF A NEW CK-M ANTIBODY AND ITS INTEGRATION INTO A LIQUID "DOUBLE SIGNAL" CK-MB TEST

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Besides Myoglobin and Troponins the activity of creatine kinase (CK) isoenzyme CK-MB is used in the diagnosis of Acute Myocardial Infarction (AMI). CK-MB activity is determined by immunoinhibition using antibodies raised against CK-M unit. Therefore, only the activity of CK-B subunit is measured in presence of CK-M subunit.
Goats were immunised with purified human CK-MM. Antiserum was treated by heat inactivation at 56 °C for 2 h and by acid treatment (pH 5.0) for 24 h. It was further purified by ion exchange chromatography to separate serum proteins and ammonium sulfate precipitation to eliminate transferrin.
In the present study we compared the behaviour of commercially available polyclonal and monoclonal antibodies against purified CK-isoforms (Scripps Labs). Criteria were lowest inhibition time (<2 min), best capacity for isoform inhibition (>99%) and highest reagent stability (>11 days at 37 °C). As a consequence we choose the abovementioned antibody for integration into our novel CK-MB test. Moreover, we made comparison studies on patient samples using assays with different amounts of antibodies. The favoured antibody amount equals 5× the halfmaximal inhibition constant (K0.5 (CK-M)).
With new antibodies we measured lower CK-MB activities of about 10% in general and up to 50% for specific samples. We could extend the reagent stability at 37 °C from 9 to 12 days. Our 2-component assay includes determination of interfering adenylate kinase activity in a prereaction to minimise hemoglobin interference. Furthermore, we offer a liquid supplement which increases precision and sensitivity by doubling the measured signal.

TP1.75

EVALUATION OF A HIGH-SENSITIVE C-REACTIVE PROTEIN METHOD ON KONELAB ANALYSER

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As elevated values of C-reactive Protein (CRP), measured with a high-sensitive method (hsCRP), have been shown to be predictive for cardiovascular diseases, we evaluated a new hsCRP method on Konelab 60i analyser (Thermo Electron, Vantaa, Finland), and compared the method to a well-established micro-particle enhanced nephelometric method (BN, Dade-Behring, Germany) using 155 patient samples with CRP values less than 10 mg/l.
HsCRP method evaluated is an immunoturbidometric assay using a polyclonal CRP-specific antibody, conjugated to microparticles. The assay standard curve range is 0.2–10 mg/l, automatically

diluted by the analyser from a stock standard, typically 10 mg/l. The extended measurement range is up to 40 mg/l with automatic dilution. In the assay 10 μl of the sample is first incubated with 120 μl buffer for 3 min, 12 μl of the reagent is added, and the mixture is incubated for 5 min. End-point absorbance is measured at the wavelength 540 nm.
The functional sensitivity of the assay was 0.20 mg/l. The within-run precision ($n=20$) was from 1 to 2.4% (CV) for samples with CRP concentrations from 2.7 to 8 mg/l, and from 3 to 5% for samples with mean concentrations of 1 mg/l or less. The between-day precision ($n=5$) was 1.3% for a sample with a mean concentration of 2.7 mg/l and 11% for a sample with a mean concentration of 0.5 mg/l. The correlation of the two methods yielded a correlation coefficient (r) of 0.98.
The hsCRP method on Konelab analyser is accurate and precise, and it correlates well with the reference method.

TP1.76

VALIDATION OF PARTICULAR ANALYSES ON ABX PENTRA 400 IN COMPARISON WITH COBAS MIRA

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Manual analyses requiring specific adaptations for automation were tested on ABX Pentra 400 in comparison with Cobas Mira, using the same reagents. Plasma pyruvate and acetoacetate, urine citrate and oxalate were performed respectively with reagents from Roche, Sigma, Diffchamb and Trinity Biotech. Within run imprecisions CV (%) for three different levels were less than 2.79, 7.44, 5.78, 1.85, respectively, for pyruvate, acetoacetate, citrate and oxalate analysis. Between day imprecisions CVs (%) for three different levels were less than 2.81, 7.72, 9.71, 8.07, respectively, for the same tests. Linearity experiments demonstrate clinical reliable ranges of measurements respectively from: 10–1200 μmol/l, 10–1000 μmol/l, 0.1–5.2 mmol/l, 50–1100 μmol/l.
Correlations with Cobas Mira were respectively:

P400=1.081 Cobas − 12.58 for pyruvate ($n=49$) ; $R^2=0.956$
P400=0.9972 Cobas+3.5742 for acetoacetate ($n=70$); $R^2=0.9102$
P400=1.086 Cobas+0.0159 for citrate ($n=35$); $R^2=0.9833$
P400=0.7741 Cobas+18.969 for oxalate ($n=35$); $R^2=0.8157$.

In all cases, ABX Pentra 400 adaptations gave a high level of analytical performances and a good concordance with Cobas Mira results. In particular, the performance of acetoacetate, because of its sensitivity, allowed determination of low values. These initial results open the field to other blood analyses adaptations, such as beta hydroxybutyrate, total acid phosphatases, angiotensin conversion enzyme.
ABX Pentra 400 demonstrates significant improvement of sensitivity and reliability. It has been shown to be a useful and flexible tool for automation of some manual analyses.

TP1.77

EVALUATION OF A LATEX AGGLUTINATION INHIBITION ASSAY FOR HAEMOGLOBIN A1c MEASUREMENT

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Haemoglobin A1c (HbA1c) is generally considered the most reliable indicator of long-term glycaemic control because it reflects the average blood glucose concentration of the previous 2–3 months and consequently requires less frequent measurement to assess glycaemic status.
The aim of study was to evaluate a latex agglutination inhibition/colorimetric assay for HbA1c measurement on an Olympus AU2700 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany) and to compare results with those obtained with HPLC/ion-exchange method on Bio-Rad VARIANT™ II Haemoglobin Testing System. The blood samples were collected from 107 diabetic and non-diabetic subjects. For the Olympus method, after a manual haemolysis step, HbA1c and total haemoglobin are determined separately and HbA1c is expressed as a percent of total hemoglobin. Within-run variations (CVs) were: 0.96%, 0.92% and 0.60%, for mean HbA1c concentrations of 5.32%, 7.73% and 10.50%, respectively. CVs for the between-day imprecision were 2.15%, 1.99% and 1.76%, for mean HbA1c concentrations of 5.33%, 7.84% and 10.67%, respectively. Regression analysis comparing the Olympus assay (y) and HPLC method (x) yielded the following equation: $y=0.585+0.942x$ ($r=0.996$, Sy, $x=0.1808\%$). The methods were also compared by using Bland–Altman plots; the mean difference between the measured HbA1c values was −0.187%, with a 95% confidence interval of −0.145% to −0.229%.
The Olympus assay showed close agreement with an HPLC/ion-exchange method. It provides a simple, rapid and precise procedure for estimating of HbA1c that does not require expensive, dedicated instrumentation.

TP1.78

DEVELOPMENT OF A 2-COMPONENT PHOSPHATE ASSAY WITH ACTIVE CLEARANCE OF LIPID TURBIDIMETRY

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In clinical chemistry phosphate is determined by the well established molybdate UV-method. So far this assay is used as a single reagent taking advantage of its great simplicity but accepting interference by lipids. Turbidity of samples due to high lipids reduces the range of measurement because of high level blank signal. Such very high levels often cannot be compensated using bichromatic measurement, therefore, assays exhibit a limited measurement range. Typical values for commercially available assays are ranges between 0.5 and 20 mg/dl Phosphorous.
We developed a 2-component system which solubilises lipids by a combination of nonionic detergents. The clearing system is

balanced to remove lipids by polyethylenglycol ether of different alkanols. The alkyl chain of such substances determines the character of the detergent. The hydrophobic component is responsible for fast clearing in the prereaction of reagent R1. Because hydrophobic detergents develop micelles are dependent on temperature, the absorbance of reagent may change during measurement. A hydrophilic component is used to avoid such turbidity of the hydrophobic component at temperatures less than 40 °C. Within 3 min of the pre-reaction unspecific absorbance is reduced to nearly the same level as without lipids. Care must be taken to reach a stable level before the assay is started by reagent R2.

Active clearance by detergents allows considerable increase of the measuring range for phosphate tests. Our novel reagent solubilises lipids up to 2000 mg/dl. No turbidity of detergents was measured up to 45 °C. Consequently, the assay shows linearity up to 40 mg/dl without changing the advantages of the monoreagent.

TP1.79

THE USE OF REFERENCE METHOD VALUES IN THE COMPARISON OF PROGESTERONE ASSAYS IN THE UK (2003–2005)

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WEQAS is one of the largest EQA providers in the UK with over 600 participants. Post market vigilance of the implementation of the IVD Directive is an essential function of EQA organisations. Traceability of results to the SI unit utilising reference target values is the preferred method of comparison of returned results where available, ensuring the transfer of accuracy from definitive methods to routine methods. Six pools of human serum, encompassing the analytical range for progesterone, with 2 endogenous and 6 spiked, were analysed by a validated reference method utilising Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS). The methodology was based on a published progesterone reference method and satisfied all published analytical criteria. Traceability of analysis was assured by the inclusion of certified reference material within the analytical run.

The prepared pools were circulated over an 18-month period to all 25 participants of the WEQAS endocrine scheme. The deviations from the 'true' result (the reference method) for the main analyser groups were plotted in the form of Bias plots (Bland–Altman plots). The Bayer Immuno 1, Roche E170 and Advia Centaur showed good agreement with the reference values. The Beckman Access, DPC Immulite and ACS 180 showed good agreement up to 25 nmol/L but samples over 60 nmol/L showed a negative bias. The study highlights the usefulness of reference targeted data as an accuracy target in EQA Schemes.

TP1.80

EXAMINATION OF URINARY ELEMENTS BY IQ 200

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There is a need for automation in urinary elements examination.

Comparison of erythrocyte and leukocyte examination by using automatic urine microscopy (iQ 200, Iris Diagnostics), flow cytometry (UF-100 Sysmex) and dipsticks (Urisys 2400, Roche) was performed. Spearman correlation coefficients in erythrocyte measurement were: iQ vs. UF-100 $r=0.682$, iQ 200 vs. Urisys $r=0.689$, UF-100 vs. Urisys $r=0.584$. Spearman correlation coefficients in leukocyte measurement were: iQ 200 vs. UF-100 $r=0.882$, iQ 200 vs. Urisys $r=0.882$, UF-100 vs. Urisys $r=0.787$. Differences of specificity between iQ 200 and UF-100 in erythrocyte measurements are 17% (Iris manual) and 30% (experimentally obtained). Differences of sensitivity between iQ 200 and UF-100 in erythrocyte measurement are 10% (Iris manual) and 12%(experimentally). Differences of sensitivity in leukocyte measurement are 5% (Iris manual) and 2% (experimentally), differences of specificity are 10% (Iris manual) and 6%(experimentally). Optimal cut-off values, estimated by ROC analysis are 10/ul erythrocytes and 15/ul leukocytes.

We can conclude that agreement between iQ 200 and dipstick method is clearly higher than between UF-100 and dipstick and also clearly higher in leukocytes than in erythrocytes. Manufacturer's data on the specificity and sensitivity were successfully verified except data on erythrocyte measurement specificity. Also, the number of samples for manual verification after analysis on the iQ200 is significantly lower (<1%} than in the case of UF-100 (approx. 25%).

The main reasons for using iQ 200 in laboratory are as follows: decrease of turn around time, reduction of preanalytical variability, possibility of regular internal quality control, exclusion of subjective influences in measurement, flexibility of system due to using the self educated neural net.

TP1.81

A NEW AND IMPROVED OLYMPUS CK NAC ASSAY ON THE OLYMPUS AU400™, AU640™ AND AU2700™ CHEMISTRY IMMUNOANALYZERS

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Aims: Evaluation of a new Olympus three-part liquid-stable automated quantitative assay for Creatine Kinase (OSR6179/6279) in human serum and plasma on the Olympus AU400, AU640 and AU2700 chemistry immunoanalysers.

Methods: The new CK NAC OSR6179/6279 assay consists of 3 parts. The R1 reagent is split into 2 parts, R1-1 and R1-2, which are combined before placing on-board the instrument to give the working R1 reagent. The on-board stability of this combined working R1 reagent remains unchanged. There is no change to the R2 reagent.

Results: Splitting the R1 reagent into two parts results in an increase in the shelf life of the CK NAC assay from 6 months to 18 months. The shelf life is increased because the sensitive enzyme activator is now in the R1-2, making it free from unwanted side reactions with other raw materials. This sensitive enzyme activator is a critical component of the assay as it

ensures full catalytic activity of the CK molecule in serum and plasma.
The method comparison of the two-part method to the new three-part method showed the following correlation:
$Y=0.992x+0.026$; $r=0.1.000$; $n=109$; range: 22–1903 U/L.
Other performance characteristics of the improved assay such as control recovery, interference (lipaemic, icteric and haemolytic), precision, linearity and LDL have been verified as unchanged from the reference two-part assay.
Conclusions: The improved CK NAC assay displays a longer shelf life whilst maintaining all previously claimed performance characteristics.

TP1.82

PERFORMANCE OF CORTISOL ASSAYS IN THE UK – COMPARISON WITH REFERENCE METHOD

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WEQAS is one of the largest EQA providers in the UK with over 600 participants. Post-market vigilance of the implementation of the IVD Directive is an essential function of EQA organisations. Traceability of results to the SI unit utilising reference target values is the preferred method of comparison of returned results where available, ensuring the transfer of accuracy from definitive methods to routine methods. Fifteen pools (pools 58–72) of human serum, 10 endogenous and 5 spiked, encompassing the analytical range for cortisol (150–1100 nmol/L), were analysed by a validated reference method utilising Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS). The methodology was based on a published cortisol reference method and satisfied all published analytical criteria. Traceability of analysis was assured by the inclusion of certified reference material within the analytical run.
The prepared pools were circulated over an 18-month period to all participants of the WEQAS endocrine scheme. The deviations from the 'true' result (the reference method) for the main analyser groups were plotted in the form of Bias plots (Bland–Altman plots). All of the analysers (ACS 180, Tosoh AIA, Bayer Centaur, Immuno 1, Roche E170 and DPC Immulite) showed a positive bias of results over the analytical range for endogenous samples. The only analyser showing good agreement for spiked samples was the Immuno 1, with all others again showing a positive bias, albeit not as pronounced as for the endogenous samples. The study highlights the usefulness of reference targeted data as an accuracy target in EQA Schemes.

TP1.83

BIOMOLECULE CONJUGATES OF UP-CONVERTING PHOSPHOR LABELS

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Autofluorescence of biological samples is a critical limitation in fluorescence-based bioaffinity assays. Up-converting (UPC) phosphors are new type of labels to avoid autofluorescence without time-resolved detection. These phosphors have a unique property of converting low energy infrared radiation to visible light. For use in bioaffinity assays biomolecules must be conjugated to UPC-phosphors, which originally do not have functional groups suitable for conjugation on their surfaces. In this work we demonstrate the conjugation of UPC-phosphors to biomolecules.
Commercially available UPC-phosphor material was characterised by its spectral properties, fluorescence lifetime and intensity. UPC-phosphors were originally micrometer-sized particulates and were ground in a small ball-mill to submicron-sized colloidal particles. UPC-nanophosphors were coated with polyacrylic acid to introduce carboxylic acid groups on the surface and biomolecules were covalently conjugated to activated carboxylic acid groups.
The performance of the colloidal UPC-phosphors coated with biotinylated bovine serum albumin was evaluated in a simple heterogenous two-step competitive assay for biotin. To compare UPC-phosphors and long-lifetime fluorescent lanthanide labels the model assay was also performed with terbium chelate as a label. Both the instrument background and the ratio of the signal in the absence and in the excess of biotin were better with UPC-phosphor conjugate than with terbium labeled biotin. UPC-phosphor was detected directly from the surface of the dry well but terbium was measured using commercial dissociative enhancement procedure.
In the heterogenous model assay for biotin it was possible to achieve at least the same performance with the UPC-phosphor conjugate than with the long-lifetime fluorescent lanthanide labeled probe. Because of low instrument background and total absence of autofluorescence the UPC-phosphors have a large potential in bioaffinity assays.

TP1.84

NOVEL NON-COMPETITIVE HOMOGENOUS IMMUNOASSAY FOR ESTRADIOL

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Small analytes (haptens) are usually measured using competitive immunoassays, which are, however, always less sensitive than non-competitive assays. The aim of our study is to develop a novel homogenous non-competitive immunoassay for small analytes, which is much more sensitive than relative competitive assay. We used estradiol as a model analyte. Estradiol has clinical importance e.g. in fertility treatments or research in breast cancer.
The developed assay is based on fluorescence resonance energy transfer (FRET). Estradiol specific antibody fragment (Fab) labelled with a long lifetime fluorescent europium(III)-chelate acts as a donor and amino-modified estradiol labelled with a non-fluorescent quencher molecule is an acceptor. When the donor and the acceptor are in close proximity with each other energy will transfer from donor to acceptor, thus the emission of europium is quenched. First samples containing estradiol and Fab are incubated until binding equilibrium is reached. Then those Fabs that are not bound to estradiol are rapidly saturated by adding excess of estradiol-quencher. Hence fluorescence can be measured only from those Fabs attached to estradiol.

Protocol of the assay was pre-tested using streptavidin and biotin, during which we discovered that one quencher molecule can quench more than one europium (III)-chelate. Since Fab and streptavidin are approximately the same size we decided to use Fab in our assay. The developed assay has good sensitivity. When compared with optimised competitive assay (using same antibody) our assay had a ten times lower detection limit.

TP1.85

DEVELOPMENT AND EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF D-DIMER ON THE ABBOTT AxSYM® ANALYZER

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Measurement of D-dimer is used as an aid in the diagnosis of deep vein thrombosis (DVT) and pulmonary embolism. D-dimer testing is particularly useful for diagnosis when used in conjunction with clinical pre-test probability scoring as a rule out test for DVT in out-patients. We report here results from our development and evaluation of an automated assay for D-dimer on the Abbott AxSYM analyzer.

The assay utilizes microparticle enzyme immunoassay (MEIA) technology in a two-step sandwich assay format, where D-dimer present in a specimen is selectively bound by anti-D-dimer antibody-coated microparticles. Detection is achieved through an anti-D-dimer alkaline phosphatase conjugate and a fluorogenic enzyme substrate, the fluorescence intensity is measured by the AxSYM optical assembly. The assay is calibrated with six calibrators prepared from commercially sourced D-dimer antigen.

Mean analytical sensitivity was assessed with one reagent lot on one instrument and found to be <7 ng/mL Assay imprecision for three controls, with concentrations of 220 ng/mL, 600 ng/mL and 1244 ng/mL, was determined by running each control in replicates of eight on two instruments using one reagent lot. The total coefficients of variation (CV) across the 20 replicates for each control were 3%, 5% and 4%, respectively. A preliminary correlation with a commercially available assay for D-dimer resulted in $r > 0.9$.

The time to first result for the AxSYM assay is 15 min with throughput of 56 tests per hour. Based on our evaluation, we conclude that this assay is a sensitive, quick, precise and accurate method for measuring D-dimer levels in human plasma.

TP1.86

SALIVARY CORTISOL MEASURED ON AN IMMULITE ANALYSER IN JET LAG AND SLEEP DEPRIVATION

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Background: Only 5–10% of total plasma cortisol is free and biologically active; the remainder is reversibly bound to corticosteroid binding globulin (60–80%) and albumin (15–30%). The measurement of salivary cortisol, which reflects the free cortisol levels, has been increasingly used.

Objectives: (1) Optimise a method for salivary cortisol measurement on the DPC Immulite. (2) Compare morning salivary and plasma cortisol concentrations. (3) Assess salivary cortisol measurements in jet lag and sleep deprivation.

Methods: Participants included six subjects returning to the UK from the USA and five subjects working on night shifts. Baseline salivary cortisol profiles were compared with those during the days following return to the UK, or following sleep deprivation.

Cortisol was extracted from 1.3 ml of saliva into 4 ml of dichloromethane. 3 ml of the extract was dried in a vacuum oven and redissolved overnight in 130 μl of Immulite diluent. Cortisol was measured in the salivary extracts and in concurrent blood samples on an Immulite.

Results: The salivary cortisol method had an inter-assay CV of 9.6% and an intra-assay CV of 7.96%. Salivary cortisol correlated with plasma cortisol ($R^2 = 0.69$). Cortisol concentrations were decreased in the morning ($p = 0.043$) and increased at midday ($p = 0.018$) after an eastbound transatlantic flight, and decreased in the morning following a night of total sleep deprivation ($p = 0.043$).

Conclusions: Salivary cortisol measured on an Immulite following extraction into dichloromethane provides a meaningful daily cortisol profile with acceptable correlation with plasma levels. The large volume of saliva required, and the time-consuming extraction process, are disadvantages. Although salivary cortisol provides a non-invasive profile, it was not possible to monitor protocol compliance, and 18% of samples were not collected as a result.

TP1.87

EVALUATION OF CAPILLARY ZONE ELECTROPHORESIS SYSTEM VERSUS A CONVENTIONAL AGAROSE GEL SYSTEM FOR ROUTINE SERUM PROTEIN SEPARATION AND MONOCLONAL COMPONENT TYPING

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Capillary zone electrophoresis of serum proteins is increasingly gaining impact in clinical laboratories. During 2003, we compared fully automated capillary electrophoresis (CE) system from Beckman (Paragon CZE 2000, CZE) with our reference method, agarose gel electrophoresis (Sebia Hydrasis-Hyris, AGE). This study focused on the evaluation of analytical performances by a comparison study of 115 fresh routine samples (group A) and 97 frozen pathologic series with suspicion of monoclonal protein (group B).

Coefficients of variation CVs (%) for the five classical protein fractions have been reported to be consistently <9% in within-run and <11% in between-run imprecision studies with CZE system. The results of the comparison study (group A) demonstrated a good correlation between CE system and AGE, except for beta globulin ($R^2 = 0.4246$). Among the 97 pathologic serum samples (group B), there are 90 in which we detected a monoclonal protein by IF (immunosubtraction was not used). AGE and CZE failed to detect 7 and 12 monoclonal proteins, respectively—sensitivity of 92% for AGE and 87% for CZE for identifying electrophoretic abnormalities

in this group. Beta abnormalities, M paraprotein are well detected with CE.
CZE BECKMAN is a reliable alternative to conventional agarose gel electrophoresis, combining advantages of full automation (rapidity, ease of use and costs) with good analytical performances. Interpretation of results requires an adaptation period which could further improve the concordance between methods. More recently the CE system has been improved by manufacturer by changes in the migration buffer with better detection of beta globulin abnormalities.

TP1.88

UNCERTAINTY AND TRACEABILITY OF A CALIBRATOR FOR CONJUGATED BILIRUBIN AND PROBLEMS OF AN AUTOMATED JENDRASSIK–GROF REAGENT

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Due to the European IVD regulations calibrators have to be traceable to standards of higher order or to a reference method. To make traceable our in-house DCA reagent and calibrator we selected the method of L. Jendrassik and P. Grof as described by G. Schellong and U. Wende (1960). On a spectrophotometer we determined conjugated bilirubin in 20 human samples (0.14–8.6 mg/dl) by manual procedure. On the same system we determined the samples with our DCA reagent using a commercially available calibrator (TruCal U). Correlation data indicated a slope of 1.21. We adjusted the value of the calibrator from 2.73 to 2.26 mg/dl. Using this value we obtained: $Y(\text{DCA}) = 1.00$ (ref. method) $+ 0.11$; $r = 0.999$. Total uncertainty for the calibrator was calculated as 5.2%.
We then did a comparison study of our DCA reagent to a commercially available Jendrassik–Grof reagent designed for use on fully automated analyzers (Hitachi 911). For DCA we used the new calibrator and for the other reagent the calibrator as assigned. Correlation ($n = 108$); $Y(\text{Jend.–Grof auto.}) = 1.1(\text{DCA}) - 0.073$, $r = 0.845$. Data analysis indicated many negative results for the automated Jendrassik–Grof reagent. The discrepancy was further evaluated by linearity studies. The results revealed that the automated Jendrassik–Grof reagent displayed problems in linearity. These become critical if a broad measuring range is claimed and the calibrator concentration is set too high. Overestimation at high concentrations and underestimation at the low end could be explained.
Summary: A calibrator for conjugated bilirubin has been made traceable to the Jendrassik–Grof method by measurements of selected human samples. A linearity problem in a commercial assay may lead to falsely negative values.

TP1.89

EVALUATION OF THE SEBIA URINARY PROFILING SYSTEM: HYDRAGEL PROTEINURIA/BJ

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Introduction: Protein profiling is generally based on molecular weight marker proteins pointing to glomerular or/and tubular proteinuria. Screening for the presence of M-components in urine, especially of Bence-Jones proteins by immunofixation is very important for diagnosis and follow-up of patients with haematological malignancies or under immunosuppression.
Methods and Materials: We evaluated a new technique allowing protein profiling and identification of monoclonal components simultaneously, on unconcentrated urine and compared the results with the conventionally used qualitative electrophoresis and immunofixation technique and with nephelometric quantification of the marker proteins.
Sixteen urines were analysed by nephelometry (BNII Dade Behring/Belgium), Protur/Microprotur (Beckman Analis/Belgium), immunofixation (Sebia Benelux/Belgium) and Hydragel-Proteinuria/BJ (Sebia Benelux/Belgium).
Results: Protein Profiling: Classification based on the Urinary Protein Profiling-system showed excellent agreement with the Beckman system and with nephelometry.
Screening for M-components: In 2 urine samples, Bence Jones could not be detected by the SEBIA-system, nor by the Beckman-system, but only by immunofixation.
More M-components were detected by both electrophoretic systems as compared to nephelometric quantification. The kappa/lambda ratio and quantification of immunoglobulins in general are not sufficient to reveal the presence of potential M-components.
Conclusion: Protein profiling by electrophoresis/immunofixation is more indicated as a first screening test because detection of M-components is more accurate as compared to nephelometry.
The SEBIA Urinary Profiling System is very appropriate as protein profiling technique. As protein profiling and identification of potential M-components are performed simultaneously, and no concentration of urine is necessary, turnaround times are much shorter.
Quantitative techniques are less suitable to reveal the presence of M-components but are very useful for follow-up and more objective as compared to the qualitative electrophoretic systems.

TP1.90

ANALYTICAL PERFORMANCE OF IMMUNOCHEMILUMINESCENT REAGENTS FOR TESTOSTERONE MEASUREMENT WITH THE LIAISON SYSTEM

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Testosterone, the main androgen produced by the Leydig cells of the testis, is a steroid hormone of molecular weight 288.4. In this study we evaluated the analytical performances of immunochemiluminescent reagents for testosterone measurement with the fully automated system Liaison (DiaSorin, Saluggia VC–Italy).

Precision was tested according to a modification of NCCLS EP-15 protocol: 14 proficiency samples were analyzed in replicates of 4 each day for 5 days.
Functional sensitivity was determined from precision testing of low concentration samples: eight samples were analyzed in replicates of four in five separate runs. A best fit regression (non-linear) was performed and the functional sensitivity calculated as the concentration where the regression line crosses 20% CV.
Dilution linearity was estimated with a linear regression of the observed vs. expected values for six samples diluted with an appropriate diluent in five different ratios. The neat sample and each dilution were assayed in replicates of four.
Recovery was determined according to NCCLS protocol, by sample addition.
Method correlation was determined by analysis of 100 samples assayed by Liaison, Immulite 2000 (DPC, Los Angeles) and ADVIA Centaur (Bayer, Tarrytown NY) on the same day, results plotted in linear regression and Bland Altman analysis.
Results: sample concentration for precision study ranged between 0.7 and 10.4 ng/mL and CV observed were under 10% and 15%, respectively, for intra- and inter-assay. Functional sensitivity was 0.6 ng/mL and regression of observed vs. expected concentration in dilution linearity was $y=1.12x+0.4$ with $R^2=0.993$. Mean recovery was 99%. Non significant differences for results obtained by Immulite 2000 ($p=0.115$, $y=1.12x+0.4$ $R^2=0.972$) and ADVIA Centaur ($p=0.549$, $y=0.98x+0.3$ $R^2=0.980$).

TP1.91

COMPARISON OF DETERMINATIONS FOR ENZYMES MEASURED ON THREE BIOCHEMICAL ANALYSERS: HITACHI 912, OLYMPUS AU 400 AND ADVIA 1200

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Introduction: Assessment of liver and cardiac enzymes is required before treatment of cancer patients. There are a lot of biochemical analysers used in routine daily practice, and the results should be comparable between different laboratories and independent of the individual manufacturers' reagent kits.
The aim of our study was to evaluate both the biochemical analysers and also reagents kits supplied by their producers.
Material: The study used sera obtained from 144 patients suffering from cardiac disease and cancer. Quality control was performed in compliance with the recommendations of firms using commercially available control materials.
Results: The serial precision of enzyme determinations obtained on 3 analysers, calculated as coefficient of variation, complied with that stated by manufacturers, whereas values of inter-assay precision were in some instances higher than those quoted by producers of reagents kits. Values of the relative error (%) for the normal and pathological levels were within the ranges of acceptable error limit. The lowest accuracy was obtained for determinations of ALP, ALT and AST. The results for all enzymes obtained with Roche Diagnostics reagents, in determinations of normal and abnormal specimens showed correlation coefficients $r>0.99$ when compared with both Bayer and Olympus reagents. Passing and Bablok regression indicates no significant deviation from linearity for all tests excluding ALP and CK NAC. The Mountain plot provides information about the distribution of the differences between determinations of enzymes in the 3 measuring systems.
Conclusion: All coefficients of correlation were high and comparable with results obtained on three analysers.

TP1.92

CLINICAL EVALUATION OF QUANTIA D-DIMER, A NEW ASSAY FOR THE ABBOTT CLINICAL CHEMISTRY SYSTEMS

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Objective: From the large number of patients presenting to the Emergency Departments (ED) with symptoms of Venous Thromboembolism (VTE), only a few are confirmed positive. Diagnostic algorithms, usually starting with a clinical evaluation followed by D-Dimer testing, aim to ease and speed the time to decision, minimising the need for imaging tests while keeping or even increasing the accuracy and safety of the diagnosis. We evaluated Quantia D-Dimer, a latex immunoturbidimetric assay, on the AEROSET® (Abbott) and compared it to HemosIL D-Dimer on the ACL TOP (Instrumentation Laboratory) and Vidas D-Dimer (bioMérieux).
Method: 99 samples from individuals presenting symptoms of VTE (32 confirmed VTE positive) were analysed with the three methods.
Results: The comparison (Passing and Bablok) of Quantia D-Dimer to the other assays yielded the following results. HemosIL as reference: slope 0.95, intercept 0.5; r 0.935; VIDAS as reference: slope 0.42, intercept 2.9; r 0.883. VIDAS reports in FEUs instead of ng/mL, hence the low slope value obtained.
ROC analysis against the VTE diagnosis yielded similar AUC values: 0.895 (CI 0.832–0.957), 0.876 (CI 0.809–0.943) and 0.900 (CI 0.840–0.961) for Quantia, HemosIL and VIDAS, respectively. Quantia® D-Dimer showed, at the upper limit of the reference interval declared by the manufacturer (198 ng/mL), 100% sensitivity and NPV. The cut-off could be raised up to 250 ng/mL still with 100% sensitivity and NPV (specificity of 40.3%).
Conclusion: Quantia® D-Dimer showed acceptable correlation to HemosIL and VIDAS, with similar diagnosis accuracy as demonstrated by ROC analysis. Quantia D-Dimer is fast, fully automated and therefore well suited for ED.

TP1.93

THE ADVANTAGES OF SERUM PROTEIN SEPARATION BY CAPILLARY ZONE ELECTROPHORESIS

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In this study we compared electrophoresis separation of serum proteins by the capillary zone system (Sebia, France) with the established agarose gel electrophoresis separation method by the same company.
Samples from 637 patients were used to compare the results of these two methods, while the commercial control serum (Sebia Ref. No 4785) was used to determine imprecision within run and between day.
The comparison of results of electrophoretic separation of patient's samples by described methods revealed that the correlation coefficients for specific fractions ranged from 0.639 to 0.884. The obtained results of correlation coefficients were statistically significant ($P<0.01$). Coefficients of variation (CVs) for imprecision within run using agarose gel electrophoresis were 1.4%, 3.4%, 1.3%, 6.9%, 20.0% and 3.2% for albumin, $\alpha1$, $\alpha2$, $\beta1$, $\beta2$ and γ fractions, respectively, while they were 1.0%, 3.6%, 1.8%, 2.1%, 9.3% and 3.3% for capillary electrophoresis. For between day imprecision coefficients of variation for the same fractions using agarose gel electrophoresis ranged from 1.6% to 10%, and ranged from 2.0% to 8.1% for capillary electrophoresis.
These results lead to the conclusion that the separation of serum proteins by capillary electrophoresis shows good agreement with the established method of agarose gel electrophoresis. The advantage of capillary zone electrophoresis is that it is simple, fast and efficient to perform, with a slightly lower coefficient of variation for individual fractions.

TP1.94

CHANGE FROM DCCT TO THE IFCC CALIBRATION FOR HBA1C MEANS NO CHANGE IN DIABETIC CLASSIFICATION

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The Czech Republic changed its calibration of HbA1c measurement on 01.01.2004. This change was realised by a consensus between diabetes and clinical biochemistry societies. Calibration of measurement systems was successfully performed by close cooperation between clinical laboratories and manufacturers. The external quality assessment programme has also been adapted to the IFCC calibration. At present more than 90% of Czech clinical labs use methods for HbA1c determination traceable to the IFCC reference method. The Czech diabetes society also consequently changed the cut-off values for well controlled diabetic patients from poorly controlled ones. The cut-off corresponding to the DCCT calibration (7.5%) was decreased to 6.0% (IFCC calibration).
We measured HbA1c in 248 patients by Variant II Bio Rad and Drew DC-5. Both systems use the analytical principle of HPLC ion-exchange chromatography. In all cases we calculated HbA1c results by both DCCT and IFCC calibration. After calculation by DCCT calibration using the cut-off of 7.5% our patients fell into two subgroups—well controlled and poorly controlled. After recalculations of our results by the IFCC calibration we reclassified the patient with the new cut-off. We observed classification change in only 1.7% patients (Variant II) or 3% (Drew DC-5). We also estimated expanded combined uncertainty in our measurement from reproducibility, bias and bias uncertainty. Its value was Uc=4.4% (Variant II) and 5% (Drew DC-5), respectively. In both cases the deviation in clinical classification after calibration change from DCCT to IFCC was less than combined measurement uncertainty.
We can conclude that calibration change from DCCT to IFCC in HbA1c measurement very probably does not lead to misclassification of diabetes compensation.

TP1.95

SEPARATION OF 11β-HYDROXYANDROSTERONE AND 17-HYDROXYPREGNANOLONE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GCMS). A COMPARISON OF DIFFERENT DERIVATIVES AND THEIR USE IN THE DIAGNOSIS OF BIOSYNTHETIC DEFECTS OF STEROID METABOLISM

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Urine steroid profiling allows the diagnosis of classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency from a characteristic pattern of metabolites. 17-Hydroxyprogesterone accumulates and is metabolised to various pregnanetriols and 17-hydroxypregnanolone (17OHP). To chemically stabilize steroids before GC analysis carbonyl groups are converted to methyloximes (MO) and hydroxyl groups to trimethylsilyl (TMS) ethers.
However with gas chromatography (GC) columns currently in routine use 17OHP co-elute with 11-β-hydroxyandrosterone (11OHA) a C19 metabolite of cortisol. To effect the resolution of 11OHA and 17OHP standard solutions of each steroid was oximated with benzylhydroxylamine (BO), ethylhydroxyamine (EO) and hydroxylamine (HO).
The retention times as methylene units for 11-OHA and 17-OHP were 27.00 and 27.02 for MO, 33.31 and 33.38 for BO, 27.40 and 27.5 for EO and 27.60 and 28.09 for HO. BO and EO spectra were similar in fragmentation pattern to MO. HO-TMS spectra were different from MO-TMS for several steroids. Interpretations of steroid profiles from patients with CAH were not hindered when using HO-TMS. Complete separation of the 2 important steroids was achieved with HO. Standard curves were constructed for 11OHA and 17OHP using HOTMS derivatives and analysed by GCMS. MOTMS and HOTMS derivatives were prepared from 22 patients with 21-hydroxylase deficiency at diagnosis and during treatment. Independent measurements of the two steroids were found to be useful when patients were being treated with hydrocortisone. The profile may also assist recognition of partial and non-

classical carriers for CAH and patients with ovarian sources of 17 OHP.

TP1.96

SIMULTANEOUS MEASUREMENT OF IMMUNOSUPPRESSANTS BY ELECTROSPRAY TANDEM MASS SPECTROMETRY (TMS)

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Frequent monitoring of immunosuppressants in the early post transplant period is essential for favourable clinical outcome. Some patients are on combination therapy in an effort to reduce toxicity. We currently use three separate assays. This work was under taken to consolidate the three common immunosuppressants in a single analytical method.

Tacrolimus, Cyclosporin A and Sirolimus were simultaneously measured on a Micromass Quatro micro coupled to a Waters HPLC system. The analysis was performed in positive ionisation mode using multiple reaction monitoring (MRM) of specific precursor and product ions.

Whole blood (20 μl) was mixed with 0.1 M Zinc sulphate (80 μl) and acetonitrile (200 μl) containing the combined internal standard (Cyclosporin D and Ascomycin) centrifuged and 20 μl of the supernatant was injected on to an on-line sample cartridge column (4.0 mm $\times$ 3.0 mm; C18 Phenomenax) and eluted with a step gradient using a mobile phase consisting of water, methanol, 2 mM ammonium acetate and 0.1% formic acid.

The assay was calibrated using the Chrom Systems one point calibrant.

The assay linearity for Tacrolimus, Cyclosporin and Sirolimus were 0–200 μg/L, 0–2500 μg/L and 0–100 μg/L, respectively.

Within batch/between batch imprecision were 1.8–6.2%/4.5–6.4%, 1.3–5.0%/2.6–7.4% and 3.5–9.5%/5.1–12.5% for the three drugs, respectively. Recovery was 98–104% for Tacrolimus, 72–88% for Cyclosporin and 97–111% for Sirolimus. The lower detection limits for Tacrolimus, Cyclosporin and Sirolimus were 0.3 μg/L, 8.0 μg/L and 0.6 μg/L, respectively.

Comparison using 50 samples with immunoassays (for Tacrolimus and Cyclosporin) and an established TMS method for Sirolimus showed good correlation.

We conclude that TMS is a specific and sensitive method for the simultaneous measurement of immunosuppressants.

TP1.97

MEASUREMENT OF ALUMINIUM, SELENIUM, COPPER AND ZINC IN SERUM SAMPLES USING ICP-MS

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Traditionally the measurement of trace elements in biological specimens has required separate analysis for each element, which can be time consuming. With inductively coupled plasma-mass spectrometry (ICP-MS) many elements can be measured simultaneously. This is useful in routine use as an increasing number of samples request a profile of trace elements, for example in monitoring patients receiving parenteral nutrition.

We have developed a method to measure aluminium (Al), selenium (Se), copper (Cu) and zinc (Zn) in serum using ICP-MS. The method involves a 20-fold dilution of sample in 1% nitric acid, using beryllium and gallium as internal standards. Several isotopes of Se, Cu and Zn were measured to determine the most appropriate for routine use. The assays were calibrated using standard addition to a serum pool.

Measurement of Se using ICP-MS has proved difficult due to an interference with argon dimers that has required collision cell technology to remove. However we have avoided this by choosing an isotope of Se less prone to interference and by optimising conditions to minimize dimer formation.

To evaluate the method a series of NEQAS specimens was analysed. The mean difference $\pm$S.E.M were -0.06 ± 0.03 μmol/L for Al, 0.10 ± 0.03 μmol/L for Se, -0.63 ± 0.16 μmol/L for Cu and -0.13 ± 0.13 μmol/L for Zn. Spike recoveries were in the range 95–120%.

The use of ICP-MS allows accurate determination of several trace elements on a single sample, reducing the analysis time from several days to several hours.

TP1.98

ANALYTICAL AND CLINICAL VALIDATION OF THE 25-HYDROXY VITAMIN D ASSAY ON THE LIAISON AUTOMATED ANALYSER

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25-hydroxy vitamin D (25OHD) measurement provides useful assessment of an individual's vitamin D stores as it has a half-life of 3 weeks. It is becoming recognised that low 25OHD levels are associated with a growing number of diseases, not only osteomalcia, rickets, secondary hyperparathyroidism and muscle disorders, but also with congestive cardiac failure, and cancers of the prostate, breast and colon. Consequently there is a continuing increase in the number of requests for 25OHD. Conventional RIA and ELISA methods are not ideal for these heavy workloads. Here we report the evaluation of a rapid automated 25OHD method on the LIAISON analyser.

The method evaluation included determination of within- and between-batch imprecision, detection limit and establishment of a reference range. Results obtained with this method were compared to those obtained with the Diasorin RIA kit and with NEQAS ALTM. Dilution studies were done 3 samples.

The within- and between-batch imprecision was determined at 2 levels (low and high) and varied between 12 and 15% within-batch $n = 12$ and 13 and 21% between-batch $n = 18$.

Comparison of results on 80 samples obtained by using this method with results using the Diasorin RIA kit gave the following equation: $Y = 0.98 + 1.11$ showing excellent correlation between the two methods. Dilution experiments showed linearity to be valid over

the range 60–176 nmol/L. There was good correlation with the ALTM for NEQAS samples.
The reference range, determined by analysis of 50 adult males and 50 adult females between the ages of 18 and 60 years, collected in August) was 26–155 nmol/L.

TP1.99

ANALYTICAL PERFORMANCES OF IMMUNOCHEMILUMINESCENT REAGENTS FOR PROGESTERONE MEASUREMENT WITH THE LIAISON SYSTEM

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Progesterone is the most potent natural progestogen and is secreted by the granulosa and theca cells of the corpus luteum under the influence of LH. In this study we evaluated the analytical performances of immunochemiluminescent reagents for progesterone measurement with the fully automated system Liaison (DiaSorin, Saluggia VC–Italy).
Precision was tested according to a modification of NCCLS EP-15 protocol: 14 proficiency samples were analyzed in replicates of 4 each day for 5 days.
Functional sensitivity was determined from precision testing of low concentration samples: eight samples were analyzed in replicates of four in five separate runs. A best fit regression (non-linear) was performed and the functional sensitivity calculated as the concentration where the regression line crosses 20% CV.
Dilution linearity was estimated with a linear regression of the observed vs. expected values for six samples diluted with an appropriate diluent in five different ratios. The neat sample and each dilution were assayed in replicates of four.
Recovery was determined according to NCCLS protocol, by sample addition.
Method correlation was determined by analysis of 100 samples assayed by Liaison, Immulite 2000 (DPC, Los Angeles CA) and ADVIA Centaur (Bayer, Tarrytown NY) on the same day, results plotted in linear regression and Bland Altman analysis.
Results: sample concentration for precision study ranged between 2.6 and 30.2 ng/mL and CV observed were under 10% and 15%, respectively, for intra- and inter-assay. Functional sensitivity was 1.1 ng/mL and regression of observed vs. expected concentration in dilution linearity was $y=1.0x+0.3$ with $R^2=0.987$. Mean recovery was 108%. Non-significant differences for results obtained by Immulite 2000 ($p=0.436$, $y=1.13x+0.7$ $R^2=0.962$) and ADVIA Centaur ($p=0.681$, $y=0.97x+0.3$ $R^2=0.958$).

TP1.100

CAPILLARY ELECTROPHORESIS WITH DIRECT UV-DETECTION OF REDUCED/OXIDIZED GLUTATHIONE AND FREE AMINO ACIDS WITHOUT DERIVATISATION

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Reduced/oxidised glutathione (GSH/GSSG) is an important peptide redox system and thiol-group containing amino acids are involved in the antioxidant defense system of various cells, protecting proteins from peroxidative injury. In the present study an efficient method permitting simultaneous separation of GSH and GSSG, as well as homocysteine, homocystine and some other amino acids/peptides compounds, employing free zone capillary electrophoresis was developed. It was achieved without prior derivatisation, employing an 8 mmol/L sodium phosphate run electrolyte adjusted to pH 2.6 and a 57 cm fused-silica capillary (50 μm ID, 50 cm effective length). Capillary electrophoresis separations were carried out in a normal polarity made at 10 kV voltage and 220C, on a Beckman P/ACE 5100 system. UV detection at 200 nm was used. Under these conditions the time needed for the GSH and GSSG separation was less than 25 min while the other examined compound migrated faster. Also complete separation of carnosine, histidine, homocysteine and homocystine was achieved, while phenylalanine/tyrosine as well as cysteine/cystine co-migrated. Linear responses were obtained in the concentration range of 10–100 μmol/L with the linear correlation coefficient of 0.932 and 0.953, respectively. The relative standard deviations of the migration times and the peak areas were found to be 0.5–3.4% and 7.1–11.4%, respectively. The method was tested on human plasma samples and whole blood haemolysate, deproteinised with absolute ethanol.

TP1.101

SIMULTANEOUS DETERMINATION OF ANDROSTENEDIONE AND TESTOSTERONE BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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The measurement of androstenedione and testosterone is useful in evaluating female patients with hirsutism, acne or signs of virilization. A sensitive, quantitative method is required for this group of patients.
Aim: To develop an assay to quantitate serum concentrations of both androstenedione and testosterone, simultaneously, using tandem mass spectrometry after liquid–liquid extraction.
Method: Serum samples or calibrators (200 μl) were prepared by adding internal standards: deuterated androstenedione (0.01 mg/L) (10 μl) and deuterated testosterone (0.02 mg/L) (10 μl) and 1 ml methyl-tertiary-butyl-ether, in eppendorf tubes containing anti-bumping granules. After vortex mixing, the supernatant was transferred into a glass test-tube, and evaporated. The residue was reconstituted using (100 μl) 50:50 mobile phase, and placed in a 96-deep well plate. 50 μl of the sample was injected into the LCMS/MS system. The retention times were 1.8 min for androstenedione and its deuterated form, and 2.3 min for testosterone and its deuterated form. Cycle time was 3.5 min, injection to injection. The analytes were monitored using a Quattro micro tandem mass spectrometer, operated in multiple-reaction-monitoring mode, using the following transitions:

androstenedione m/z 287.3>97.0, d7-androstenedione m/z 294.4>99.9, testosterone m/z 289.3>97.0, d2-testosterone m/z 291.3>99.0.
Results: The assays were linear to 50 nmol/L, the lower limit of quantification was 0.25 nmol/L, and within and between batch CV and bias were <15% for both androstenedione and testosterone. Mean recovery was 98.5% for androstenedione, and 92.7% for testosterone. Sample extracts were stable for at least 14 h.
Conclusion: We have developed a simple, accurate, precise, and sensitive LCMS/MS method, for the simultaneous measurement of androstenedione and testosterone.

TP1.102
COMPARISON OF A METHOD FOR ANDROSTENEDIONE AND TESTOSTERONE USING LC-MS/MS VERSUS IMMUNOASSAY
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Testosterone concentration is routinely requested in the investigation of oligo- and amenorrhoea, hirsutism and/or acne. The addition of androstenedione is helpful in excluding ovarian or adrenal pathology. A sensitive and accurate method to measure these androgens is required for the accurate assessment of these patients, to evaluate the need for further investigation.
Aim: To compare methods routinely used, with a method by LC-MS/MS that simultaneously determines both androgens.
Method: 92 patients, with a range of androstenedione concentrations, were analysed by LCMS/MS after sample preparation with liquid/liquid extraction, (previously validated) and by Radioimmunoassay (RIA) using Diagnostic Systems Laboratory-3800 ACTIVE. 129 patients, with a range of testosterone concentrations, were analysed by LC-MS/MS after liquid/liquid extraction, and by immunoassay using Roche Elecsys E-170 module.
Results: Comparison of LC-MS/MS against RIA for Androstenedione using Passing Bablok regression analysis showed LC-MS/MS=$0.575 \times \text{RIA} - 0.207$ $R^2=0.74$. Bland Altman analysis showed poor agreement between LCMS/MS and RIA with a bias of -1.966.
Comparison of LC-MS/MS against immunoassay for Testosterone using Passing Bablok regression analysis showed LC-MS/MS=$0.984 \times \text{Roche} - 0.051$ $R^2=0.97$. Bland Altman analysis showed reasonable agreement between LCMS/MS and immunoassay with a bias of -0.216.
However, when those samples with testosterone concentrations less than 3nmol/L were examined separately, Bland Altman analysis showed a bias of -0.334 and Passing Bablok regression analysis showed LC-MS/MS=$0.729 \times \text{Roche} + 0.087$ $R^2=0.23$.
Conclusion: RIA substantially overestimated androstenedione concentration. LC-MS/MS showed reasonable agreement with the testosterone immunoassay, but again there was overestimation by immunoassay, particularly at lower concentrations of testosterone. These findings are particularly significant for female samples, in evaluating the need for further investigation.

TP1.103
UP-CONVERTING PHOSPHORS AS LABELS IN BIOAFFINITY ASSAYS
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Improved labels are needed in diagnostics and biomedical research for sensitive, multiplexed and miniaturised ligand binding assays. Up-converting (UPC) phosphors are a new type of label, which convert infrared excitation light to emission at visible wavelengths, enabling measurements without autofluorescence. In this work potential of these phosphors as labels in solid phase bioaffinity assays was studied.
Commercial, micrometer-sized UPC-phosphors were first bead-milled to colloidal, submicron-sized particles and conjugated to biomolecules. Linearity and the limit of detection of the phosphor label were determined in comparison with europium chelate. UPC-phosphor and europium chelate bioconjugates were utilised in a solid phase bioaffinity assay to study the possibility to measure anti-Stokes photoluminescence and time-resolved fluorescence simultaneously from a single microtitration well.
Linearity of the detection was over four orders of magnitude for both UPC-phosphor and europium chelate. Lower limits of detection were roughly 1×10^4 UPC-phosphor particles and 9×10^7 europium chelate molecules in solid phase measurements in transparent microtitration wells. In white wells 50-fold improvement was detected in the detection limit of UPC-phosphor because of unexpectedly strong signal enhancement of the phosphors in these wells. This signal enhancement could not be explained by the improved light collection efficiency due to white surface, since similar enhancement was not detected with europium labels in white wells. Anti-Stokes photoluminescence of UPC-phosphor and time-resolved fluorescence of europium chelate were measured from the same well with no effect on each other's signals.
Large dynamic range and detectability comparable to europium chelate, minimal background fluorescence and signal enhancement render UPC-phosphors very attractive labels. Non-existent crosstalk between UPC-phosphors and europium chelates enables their simultaneous use in multiplexed assays.

TP1.104
ROCHE ELECSYS PROLACTIN ASSAY; INITIAL EVALUATION OF A REFORMULATED ASSAY MINIMISING REACTIVITY WITH MACROPROLACTIN
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Background: Macroprolactin (MPRL) is a cause of elevated total serum prolactin (PRL) in immunoassays but seems to have no relevant bioactivity in vivo. Manufacturers have been urged to minimise the reactivity of PRL assays with MPRL. We evaluated a prototype Elecsys PRL assay (PRL II) employing antibodies and reaction conditions formulated to minimise reactivity with MPRL, and compared results with those from existing Elecsys assay (PRL I) and with the Bayer ADVIA Centaur assay.

Methods: PRL I and PRL II were measured on Roche Elecsys 2010 and E170 systems. Samples showing a recovery <60% after PEG precipitation with PRL I were classified as containing MPRL.
Results. Between run CV for all assays was <4.7%. Linear regression analysis of correlations of results from PRL II(y) and PRL I(x) using samples without MPRL (PRL range(x): 24–24700 mIU/L, n=375) yielded slopes from 0.81 to 0.83 and between PRL II(y) and Centaur(x) slopes were 1.13–1.23. Using samples containing MPRL (PRL range(x): 300–6700 mIU/L, n=89) PRL II(y) showed similar overall reactivity to MPRL as Centaur(x) with slopes of 1.18 and 1.19 but 10 samples were outliers in the correlation showing considerably lower reactivity with MPRL in the PRL II assay.
Conclusions: The prototype Elecsys Prolactin II assay reacts less strongly with MPRL than the current PRL I assay. Introduction of PRL II into routine laboratory practice would result in fewer samples with elevated PRL due to MPRL requiring screening for macroprolactin by PEG precipitation and better agreement with the all laboratory trimmed mean in the United Kingdom External Quality Assessment Scheme.

TP1.105
CLINICAL AND LABORATORY ASPECTS OF 13C-BREATH TEST EVALUATED BY INFRARED SPECTROMETRY (NDIRS)
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Modern, non-invasive methods analysing 13C/12C ratio are available for the dynamic tests of gastrointestinal functions.
This study summarises pre- and post-analytical aspects of the 13C-breath-test (BT) evaluated by means of isotope selective non-dispersive infrared spectrometry (NDIRS)–Isomax 4000 (Isodiagnostika). Moreover, the sources of inaccuracy in test results are identified: (a) uncertain baseline 13C abundance, (b) inaccuracy of the spectrometer, and (c) uncertainty in CO2 production, which also burden cumulative BT where IR/IRMS measuring instruments are used.
Regarding (a), an estimate is presented that is closer to reality than the commonly used PDB standard. To address (b), the accuracy of measurements is assessed by a statistical analysis and by measuring IRMS calibrated samples every 4 months. After 13 cycles of checking, the calculated mean bias of the Isomax 4000 equals 5.509%. Concerning (c), two published estimates of CO2 production are used and compared: a BSA-based (Body Surface Area) estimate, and a BMR-based (Basal Metabolic Rate) estimate.
To measure gastrointestinal function, 465 BT have been performed since 2002: 53 tests with 13C-xylose, 148 with 13C-mixed triglyceride, and 264 with 13C-urea. These include 170 cumulative (7 h) 13C breath tests, particularly exocrine pancreatic tests with 13C-mixed triglyceride. The cut-off value for these pancreatic tests was calculated as the mean value of the recovery levels—2SD in a group of 35 subjects without chronic pancreatitis. It is observed that the BMR-based calculation has led to greater 13C recovery values than the BSA approach. The cause of this discrepancy is explained, and a corrected, more accurate approach is proposed.

TP1.106
EFFECT OF STRONTIUM ON CALCIUM MEASUREMENT
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Strontium in the form strontium ranelate has been approved for the treatment of osteoporosis. Strontium has been suggested to interfere with calcium methods. We have evaluated this interference in calcium measurement.
Strontium was added to 10 serum samples to give final concentrations of 0.1, 0.2 and 0.4 mol/L. Samples were analysed by the following methods:(1) indirect potentiometer in a Beckman LX20 analyser; (2) by Arenas III dye binding method in an Abbott Architect C8000 analyser; (3) by cresolphthalein complexone method in a Bayer Advia systems and (4) by ion selective electrode.
Samples with added strontium showed significantly higher serum calcium values at all levels of strontium added. Therapeutic serum strontium concentrations have been reported to be 0.2 mmol/L. At this concentration, measured calcium was higher by 0.04 mmol/L by method 1, 0.14 mmol/L by method 2, 0.115 mmol/l by method 3 and 0.04 mmol/L by method 4. The increase in calcium was linearly related to added strontium and the slope of the line was 0.238 for method 1, 0609 for method 2, 0.521 for method 3 and 0.135 for method 4. The effect of added strontium was significantly less for the indirect potentiometry and ISE methods compared to the colorimetric methods.
Strontium had no effect on measured serum magnesium concentration.
We conclude that serum calcium in patients on strontium ranelate should be interpreted cautiously.

TP1.107
MEASUREMENT OF URINE CYSTINE USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY
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Cystine is a sulphur containing amino acid. Defects in renal transport of cystine (cystinuria) lead to massive urinary excretion, and, due to its relative insolubility, nephrolithiasis. Although cystine-containing calculi are rare, raised urinary cystine excretion has also been shown to be a risk factor for the formation of calcium oxalate calculi. We developed a method to quantitate cystine levels in urine to identify patients who are at risk of recurrent nephrolithiasis.
The method involved the addition of 30 μL deuterated cystine as an internal standard to 10 μl of urine in a 96-deep well plate. 500 μl of distilled water was added to this, and the plate

vortexed. This was then placed into a Waters 2795 autosampler, and 5 μl injected onto a Phenomemex® SecurityGuard SCX column attached to a Waters™ Atlantis C18 column. The eluant was introduced directly into a Micromass™ Quattro Micro tandem mass spectrometer.

Cystine is eluted from the column after 0.89 min, a further 2 min of flow is required to re-equilibrate the column. Cystine was monitored using a transition with a mass to charge ratio (m/z) of 241.1>152, and the deuterated cystine internal standard was monitored using a transition of m/z 244.8>153.8. Ion suppression is negligible under the conditions used.

The assay was linear up to 1000 mg/L, with a lower limit of quantitation of 10 mg/L. Intra- and inter-assay precision were acceptable with CV<5% and bias <15% at all concentrations used. We have developed a quick, robust assay for the quantitation of cystine in urine that will enable patients at risk of recurrent nephrolithiasis to be identified.

TP1.108

EVALUATION OF THE EVIDENCE ANALYSER FOR DRUGS OF ABUSE ASSAYS

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The Evidence (Randox Laboratories) is a new instrument, which uses conventional immunoassay techniques but utilises antibodies attached to the surface of a biochip. The detection system uses a chemiluminescent substrate and horseradish peroxidase label, with signal detection by a camera using light sensitive diodes.

We compared urine drugs of abuse measurement by the Evidence analyser with conventional immunoassay techniques (Microgenics Corporation) on a Kone 60 analyser. Urine specimens (n=150) were screened by both methods and positive results were confirmed by capillary electrophoresis (CE) or GC-MS. Borderline or discrepant results were also subjected to confirmatory testing.

For opiate analysis, 136/150 results were in agreement by both methods. Fourteen specimens gave positive results by the Microgenics method but negative results by the Evidence analyser. All fourteen urines were negative for morphine, mono acetyl morphine, and codeine by CE or GC-MS.

For methadone analysis, 147/150 results were in agreement with the two methods. Three urines gave borderline positive results by the Microgenics method but negative results by the Evidence analyser. All three urines were positive for methadone metabolite.

For cocaine metabolite, 145/150 results were in agreement with both methods but five were positive by the Microgenics method and negative by the Evidence Analyser. All five urines were negative for cocaine metabolite on confirmatory testing.

Our data show that the Evidence analyser produced fewer false positive results when compared with a conventional immunoassay. It is easy to use and capable of analysing up to 180 specimens/h with up to 9 drugs per specimen. In conclusion the Evidence analyser has major advantages compared to conventional immunoassays for drugs of abuse screening.

TP1.109

HOW WELL DO WE MEASURE CREATININE?

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Creatinine clearance (CrCl) is the most common estimation for glomerular filtration rate (GFR) used in laboratories and relies upon creatinine measurement. Creatinine itself can be measured by a variety of methods e.g. uncompensated Jaffe, enzymatic methods, HPLC and compensated Jaffe; the latter is the most frequently used. This method is prone to interference e.g. by bilirubin, lipaemia and ketones and underestimates GFR due to tubular secretion of creatinine.

We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the analysis of creatinine in serum (10 μL) using a Waters™ Quattro Micro™ tandem mass spectrometer coupled to a Waters™ 2795 Alliance HT LC system. Chromatography was performed on a Phenomenex® SCX 4×3 mm SecurityGuard column. The method had a lower limit of quantification of 5 μmol/L and was linear to 3000 μmol/L. The intra- and inter-assay imprecision was <5% and <7%, respectively. Serum samples were analysed by uncompensated Jaffe, compensated Jaffe and LC-MS/MS methods and results compared to previous findings. We found that the compensated Jaffe method compares well to the LC-MS/MS method at concentrations below 155 μmol/L, contrary to previous findings of underestimation; we believe this to be due to a calibration error on the automated analysers. At concentrations above this value creatinine has been found to be overestimated in the literature, although we found it to be underestimated by the compensated Jaffe method and this was unaffected by the calibration.

TP1.110

EVALUATION OF THE RESTANDARDISED ABBOTT ARCHITECT OESTRADIOL ASSAY

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A restandardised oestradiol assay for the Abbott Architect was launched in October 2004. The previous assay suffered from marked dose-related positive bias in NEQAS and poor precision at low concentrations. Locally, oestradiol samples come from a wide variety of sources but the majority is from a busy IVF Unit demanding 2 h turn around time. Daily workload is ~120 samples, with values ranging from <100 pmol/L to >12,000 pmol/L, and the new Architect assay was evaluated to provide service improvement. Two hundred samples were compared in both the old and new assays including 18 NEQAS samples. Precision was assessed by daily assay of Biorad Liquichecks (n=10) and a panel of samples with values ranging from 104 to 12,017 pmol/L (n=6). The sensitivity of the assay was investigated by replicate measurements of samples with values below 112 pmol/L. Linearity was assessed by dilution of medium and high samples.

The new assay gave values approximately 25% lower than the old assay however the differences were dose related with the largest differences (50%) being noted at low and smallest (10%) at high oestradiol concentrations, respectively. NEQAS samples varied from the ALTM by −11% to +14%. In precision studies the patient samples gave %CVs between 1.6 and 5.7 and Liquichecks gave %CVs of 7.3, 2.2 and 2.5 for low medium and high values, respectively. The functional sensitivity of the assay (10%CV) is below 70 pmol/L and high concentration samples diluted parallel to the standard curve.
The reformulated Architect oestradiol assay demonstrates excellent reproducibility over a wide concentration range, provides NEQAS results close to the ALTM, has much improved sensitivity and will improve our service provision.

TP1.111

DIRECT DIAGNOSIS OF ASPERGILLUS FUMIGATUS INFECTION FROM BREATH SAMPLES

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Invasive aspergillosis is one of the most problematic infections confronting clinicians. Detection of volatile organic compounds (VOCs) has the potential to improve the specificity and sensitivity of diagnosis of this and other infections. The aim of this study was to identify a unique biomarker of *A. fumigatus* in the headspace gas of in vitro cultures and to detect the marker from breath samples of infected or colonised patients.
Gas Chromatography-Mass Spectroscopy (GC-MS) combined with Solid Phase Micro Extraction (SPME) was used to identify 2-Pentylfuran as a specific biomarker of *A. fumigatus* from cultures. Four litre breath samples were collected from patients with Cystic Fibrosis (CF), with or without colonisation of *A. fumigatus* and other pathogens and healthy volunteers. Breath samples were semi-quantitatively analysed by SPME/GC-MS for presence or absence of 2-Pentylfuran.
A total of 21 individuals was tested. 2-Pentylfuran was detected from breath samples of 4/4 patients with CF and *A. fumigatus* colonisation, 3/7 patients with CF and no microbiological evidence of *A. fumigatus* and 0/10 healthy control individuals.
To our knowledge, this is the first report describing the detection of volatile biomarkers of a pathogen resident in the lungs from breath samples.
A new generation of highly sensitive instruments offers the possibility of detecting parts per trillion (ppt), and hence identification and detection of unique biomarkers that has the potential for improving the direct diagnosis of infectious, metabolic and other diseases. This will only be achieved by combining the expertise of clinical and analytical specialists as we have done successfully in this study.

TP1.112

EVALUATION OF IFCC TRACEABLE HbA1C ASSAY ON THE OLYMPUS AU-640 ANALYSER

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The Olympus HbA1C assay is an inmunoinhibition test for quantitative determination of HbA1c. Blood samples are pre-treated with denaturant to form a haemolysate. Total haemoglobin and HbA1C are determined by separate measurements and %HbA1C is calculated automatically. Calibrator values are traceable to the IFCC HbA1c reference method. We carried out the evaluation on the Olympus AU-640 analyser and the "gold standard" for comparison was the Menarini HPLC 8121.
Method comparison (NCCLS EP9-A2): One hundred and seven EDTA anti-coagulated whole blood samples ranging from 2.5 to 11.8% HbA1C. The linear regression was $y=0.905x+1717$.
Precision (NCCLS EP5-A): Non-diabetic (mean 3.95): Within run (S.D. 0.089; CV 2.25%); Between run (S.D. 0.046; CV 1,17%); Between day (S.D. 0.075; CV 1.89%). Diabetic (mean 6.8); Within run (S.D. 0.128; CV 1.89%); Between run (S.D. 0.167; CV 2.45%); Between day (S.D. 0.190; CV 2.79%).
Linearity (NCCLS EP6-A): The reportable range for THb was determined to be 3.01 to 16.00 g/dl and for HbA1C 0.326 to 1.63. Results were accepted by Bartlett's test.
The limit of detection (LOD) of the HbA1C assay was defined as the mean concentration of an analyte-free sample +3S.D. S.D. was calculated using the values of 21 replicates of the sample. The LOD was 0.5 g/dL.
The assay has no significant cross-reactivity with labile HbA1C. Using the procedure described in the FDA glycohaemoglobin guidance document, the results varied 2.3%.
The HbA1C Olympus assay is meeting the precision and method comparison requirements by analysis of National Glycohaemoglobin Standardisation Program. The assay permits automated analysis on open systems and this study demonstrated very acceptable analytical performance on Olympus AU-640 analyser.

TP1.113

A SIMPLE SCREENING METHOD FOR DEFECTS IN PURINE BIOSYNTHETIC PATHWAY

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Two inherited metabolic defects have been described in purine de novo synthesis pathway (PDNS)—adenylosuccinate lyase and aminoimidazole carboxamide ribonucleotide transformylase deficiency. Here we present a method which allows detection all defects in the second part of the PDNS by thin layer chromatography.
We synthesized ribosides analogous to intermediates of the pathway and used two chromatographic systems for the analysis. Two-dimensional TLC was performed using cellulose plates (DC-alufolien cellulose, Merck) using isopropanol:ammonia (4:1) and butanol:acetic acid:water (4:1:1) as the solvents. One-dimensional chromatography was performed using silica plates (HPTLC-Platten Kieselgel 60, Merck) by subsequent elution in 16 mmol/L boric acid in isopropanol:butanol:water (5:3:2) and acetone:butanol:water (5:4:1). Detections were performed by orcinol-sulfuric acid reagent and diazotized sulfanilic acid (Pauly's reagent).

Potential usefulness of the method was demonstrated on samples from patient with adenylosuccinate lyase deficiency and Chinese hamster ovary cells defective in the steps of purine de novo synthesis.
Both the separation approaches allowed analysis of the species of interest; however two-dimensional TLC is necessary for confirmation of the compounds. Detection with orcinol-sulfuric acid reagent allows detection of the compounds together with other sugars important in diagnosing metabolic disorders. Detection with Pauly's reagent which gave bluish-coloured products is very selective for the compounds of interest and no interfences were observed in 300 healthy urines analysed.
TLC is a simple and effective screening tool for defects in purine biosynthetic pathway.
This work was supported by grant IGA NR/7796-3 and NR/8578-3.

TP1.114

COMPARISON STUDY OF TWO IMMUNOASSAY METHODS FOR TUMOUR MARKERS CA125 AND CA15-3. DPC IMMULITE 2000 VS. ABBOTT ARCHITECT

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The automation of immunoassay techniques along with the development of monoclonal antibodies have spectacularly improved the precision, sensitivity and turnaround time of tumor markers measurement.
In this study, the immunoassay methods of two modern random access analysers for the antigens CA125 and CA15-3, the most valuable markers of the ovary and breast cancer, respectively, are compared and the correlation between the respective results is investigated.
Serum samples from 131 patients, and the two level of BIORAD Lyphocheck multicontrol (15 replicates in 5 runs), were assayed using the DPC IMMULITE 2000 and ABBOTT ARCHITECT analysers.
The correlation between the two methods proved ($p<0.001$) to be higher for CA125 ($r=0.947$) than for CA15-3 ($r=0.812$) and was significantly improved when high concentrations (diluted samples) were excluded ($r=0.990$ and $r=0.837$, respectively). In the low concentration range, the correlation deteriorated for both markers ($r=0.860$ for CA125<40 U/ml and $r=0.789$ for CA15-3<60 U/ml). By means of clinical assessment, 9.4% of the negative (within the reference range of the method), according to IMMULITE, results of CA125 were found to be elevated by the ARCHITECT, while 12.7% of the negative, according to ARCHITECT, results for CA15-3 were found pathological to IMMULITE. The precision of the methods was estimated by the control samples measurement and the coefficient of variation ranged between 2.90% and 4.31%.
The correlation between the two methods is high for CA125 and acceptable for CA15-3, across the whole concentration range. The deviations observed are considered reasonable, given the technical specialties and the different immunochemical materials adopted by the two methodologies.

TP1.115

COMPARISON OF HPLC FLUORESCENCE, UV AND MASS SPECTROMETRY DETECTION METHODS FOR THE DETERMINATION OF TPMT ACTIVITY

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There are many published methods for determining Thiopurine s-methyltransferase (TPMT) activity, including HPLC with absorbance, fluorescence and more recently mass spectrometry (MS) detection. The advantage of MS detection is specificity, however compared to fluorescence it may lack sensitivity. There are also huge differences in equipment costs. To directly compare these methods of detection, we have optimised an existing HPLC fluorescence method for the measurement of erythrocyte TPMT activity for use with UV and MS (electrospray) detection.
Both UV and MS were of similar insensitivity compared to fluorescence detection, requiring 25 times more sample for analysis. The chromatography obtained using UV detection was poor and without the fluorescence results as a guide, it would have been difficult to identify and integrate the correct product peak.
Using the MS in scan mode (positive ions from 150–450 m/z) the product of the TPMT reaction was confirmed as 6-methylthioguanine (6-MTG), m/z 182. We have previously reported a non-enzymatic peak in the reagent blank with the same retention time as the TPMT reaction product 6-MTG. Using MS with selected ion monitoring (SIM) at m/z 182, this product was confirmed as 6-MTG.
The linearity of UV and MS detection compared to fluorescence for determining the TPMT activity of 36 patient samples was $y=0.829x+8.0736$ and $y=0.7791x+4.2793$, respectively.
LC-MS offers an alternative, though costly, detection system for use in TPMT analysis. In comparison, UV detection lacks specificity and sensitivity. Overall this study confirms fluorescence detection as the better analytical approach.

TP1.116

DEVELOPMENT OF A METHOD FOR THE MEASUREMENT OF DEHYDROEPIANDROSTENEDIONE-SULPHATE BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Dehydroepiandrostenedione sulphate (DHEAS) is a steroid that is being recognised as a potential drug of abuse in many countries, mainly due to its reputation as a hormone that may be able to retard the aging process.
The measurement of DHEAS is useful in determining medical conditions such as congenital adrenal hyperplasia and polycystic ovary syndrome. Thus a liquid chromatography–tandem mass spectrometry method has been developed to determine DHEAS concentrations in human serum.

The chromatography was performed by a Waters™ 2795 Alliance HT LC system coupled to a Mercury Fusion-RP column, fitted with a security guard column. This gave a retention time of 1.5 min for both DHEAS and the internal standard, deuterated DHEAS. The transition determined by the Micromass Quattro™ tandem mass spectrometer for DHEAS was m/z 367.3>96.7 and for the internal standard m/z 369.3>96.6.
The method was linear up to 20 μmol/L, the lower limit of detection and the lower limit of quantitation were both 1 μmol/L. The intra- and inter-batch precision were both <8% over a concentration range 1–18 μmol/L for the in house QC and <6% for the Biorad Lyphocheck QC. This method is robust and has a simple sample preparation procedure with a rapid cycle time of only 4 min.

TP2: BONE METABOLISM/BONE DISEASE/VITAMIN D

TP2.01 SELECTED FOR POSTER CLINIC

THE ASSOCIATION OF MTHFR GENOTYPE, HOMOCYSTEINE, FOLATE AND VITAMIN B12 WITH OSTEOPOROTIC RISK

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Osteoporosis is a debilitating bone disease of multifactorial aetiology, with genetic, nutritional, environmental and endocrine risk factors. We investigated the contribution of methylenetetrahydrofolate reductase (MTHFR) genotype, homocysteine, folate and vitamin B12 to osteoporotic risk, as defined by peripheral (calcaneal) bone mineral density (BMD) measurement.
328 post-menopausal women were assigned to one of three groups (Control [C], $n=110$: Osteopenic [OPN], $n=108$: Osteoporotic [OP], $n=110$), according to Peripheral Instantaneous X-ray Absorptiometry (PIXI) 'T' score. Fasting blood samples were obtained and analysed for MTHFR genotype (PCR), plasma total homocysteine (HPLC, Drew Scientific DS30) and serum foliate and vitamin B12 (competitive protein binding assay).
The groups did not differ significantly in age (mean age, years 67.6 [C], 65.8 [OPN], 68.8 [OP]). There was an increasing frequency of the MTHFR TT genotype in the three groups (C, 8.5%; OPN, 11.1%; OP, 12.7%). Plasma total homocysteine concentrations (μmol/L) were (mean (S.D.)) C, 11.97(4.85); OPN, 11.55(6.23); OP, 13.20(5.34), the concentration in the OP group being significantly higher than that in the C group ($p<0.05$). Serum folate concentrations (ng/L) were C, 9.14(4.77); OPN, 10.1(4.66); OP, 7.61(3.92), the concentration in the OP group being significantly lower than that in the C group ($p<0.01$). Vitamin B12 concentrations did not differ significantly between the groups.
This study confirms the association between the MTHFR TT genotype (conferring increased thermolability to the enzyme) and risk of osteoporosis. Whether this genotype manifests an effect through an increase in plasma homocysteine appears to be dependent upon folate status, this being a major determinant of plasma homocysteine concentration.

TP2.02 SELECTED FOR POSTER CLINIC

CORRELATIONS BETWEEN SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) AND KNEE RADIOLOGIC FINDINGS IN 33–53 YEAR OLD SUBJECTS

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COMP has been found in various joint tissues: cartilage, tendons, synovia.
The aim of this study was to investigate the correlation between S-COMP and different tissues of the knee joints.
Altogether 107 subjects (56 male, 51 female) with chronic knee pain were examined using VAS scales, knee radiography and sonography. Radiographs from the tibiofemoral and patello-femoral joints were graded by two radiologists. Soft tissues were assessed by sonography. Serum levels of COMP were measured by ELISA (AnaMar Medical, Sweden). Statistics: Spearman's correlation, regression analysis.
The distribution of serum COMP levels was different for men and women (the medians 12.1 and 9.7 U/L, respectively, $p=0.0004$).
For women, S-COMP levels were significantly correlated with age (rho=0.429, $p=0.002$) and tibio-femoral osteoarthritis (rho=0.318, $p=0.023$) but not with patello-femoral osteoarthritis. S-COMP also correlated with thickness of the quadriceps tendon (right rho=0.337, $p=0.045$, left rho=0.435, $p=0.008$) and with the thickness of the middle part of the patellar tendon (rho=0.352, $p=0.035$).
For men, only correlation with knee pain was found (rho=0.275, $p=0.042$).
Using the combinations of variables as thickness of the quadriceps tendon, thickness of the suprapatellar synovium and patello-femoral cartilage, the regression model permitted 30–48% prediction of the variability of S-COMP in women. The model did not predict S-COMP for men.
Conclusions: Average S-COMP levels were higher in men than in women.
In women S-COMP showed a weak correlation with radiographic tibio-femoral but not with patello-femoral osteoarthritis.
Sonographic investigation revealed a significant contribution of patello-femoral cartilage, tendons and suprapatellar synovium to the variability of S-COMP in women.

TP2.03 SELECTED FOR POSTER CLINIC

VITAMIN D RECEPTOR GENE BSMI POLYMORPHISM INVESTIGATION OF MIS-GENOTYPING AND ANALYSIS OF CORRECTED GENOTYPE DISTRIBUTION

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The BsmI polymorphism of the VDR gene has been widely investigated in metabolic bone disease. The discovery of a single nucleotide polymorphism (SNP) under the reverse primer binding site of the previously published primers which could give rise to mis-genotyping led to our re-examination of this polymorphism.

BsmI mis-genotyping caused by this SNP could have confounded previous genetic findings that deviated from Hardy–Weinberg equilibrium. We re-examined the VDR genotypes with a reverse primer external to the original binding site and also confirmed the presence of the Tru I polymorphism that gave rise to the mistyping, by restriction digest.

Three groups of patients attending the bone metabolism outpatients clinic and with bone mineral density (BMD) measurement data were examined, those with normal BMD ($n=218$), those with osteoporosis ($n=234$) and those receiving steroids and with osteoporosis ($n=157$). The results provided a higher number of heterozygous Bb subjects with a proportionally reduced number of BB subjects. The Chi-Squared Tests for the 3 groups were: Normals: $p=0.006$, Osteoporotic; $p=0.024$ and Steroid-induced Osteoporotic; $p=0.016$. The re-examined genotype distribution was in Hardy–Weinberg equilibrium, giving Chi-squared tests for 3 groups: normals; $p=0.89$, Osteoporotic; $p=0.83$ and steroid-induced Osteoporotic; $p=1.00$.

The SNP in the region corresponding to the reverse primer has given rise to BsmI mis-genotyping. The corrected data show no significant association between VDR BsmI and BMD.

TP2.04 SELECTED FOR POSTER CLINIC

ASSOCIATION OF PLASMA HOMOCYSTEINE CONCENTRATION, B VITAMIN STATUS AND GENETIC FACTORS, WITH BONE MINERAL DENSITY

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Recent publications suggest exposure to moderate elevations of plasma total homocysteine (tHcy) may be associated with similar clinical outcomes as seen in homocysteinuria, differing only with regard to severity and time of onset.

tHcy, Methylene tetrahydrofolate reductase (MTHFR) C677T genotype and relevant nutrient analysis (folate and vitamin B12) were performed on DNA and plasma samples collected as part of a previous study of 258 patients (213 female and 45 male) referred to Belfast City Hospital's osteoporosis clinic. Bone mineral density (BMD) measurements at the lumber spine and hip and clinical history were available for each patient.

BMD (mean ± S.E.) declined progressively from the lowest to highest homocysteine tertile at both lumber spine and hip sites (tertile I vs. III: $0.788 \pm 0.017 g/cm^2$ vs. 0.724 ± 0.017 g/cm^2, $P<0.05$ and 0.779 ± 0.016 g/cm^2 vs. 0.710 ± 0.015 g/cm^2, $P<0.02$, respectively). tHcy was significantly higher [median (95% confidence interval):15.4 (12.3–18.6) vs. 12.9 (10.8–16.9), $P<0.005$], and serum folate significantly lower [4.57 (3.16–6.70) vs. 5.64 (3.71–9.16) $P<0.02$] in osteoporotic compared with age-matched non osteoporotic patients. There was no significant difference in B12 status [364 (261–513) vs. 357 (268–461) $P>0.05$], and there was no relationship between MTHFR C677T genotype and BMD, even in those with low folate status.

We conclude that osteoporosis is associated with elevated tHcy which in turn appears to relate to low folate levels. To our knowledge this is the first occasion that tHcy has been directly linked with both low BMD and osteoporosis.

TP2.05 SELECTED FOR POSTER CLINIC

THE EFFECTS OF NUTRITIONAL REHABILITATION ON BONE REMODELLING IN ANOREXIA NERVOSA

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Osteoporosis has been described in association with Anorexia Nervosa (AN). Protein-energy malnutrition adversely affects skeletal mass. The aim of the study was to assess the effect of nutritional rehabilitation on bone metabolism in AN, using indices of bone remodelling and turnover derived from bone formation and resorption markers.

Nine females were admitted to the Psychiatric Unit, body mass index (BMI) 12.92 ± 1.66 vs. 16.4 ± 1.48 kg/m^2* on refeeding (*$p<0.05$). The bone formation marker Procollagen type 1 N propeptide (P1NP), the best marker of collagen synthesis, and resorption markers Deoxypyridinium Crosslinks (FDPD), *N*-telopeptide Crosslinks (NTX) and β Crosslaps (βCTx) were measured. Bone Remodelling Balance Index (BRBI)(formation-resorption) and Bone Turnover Index (BTI)(formation+resorption) were calculated. Results were expressed as *T* values (observed value − mean reference range/S.D. reference range). Normal BRBI = ±2, BTI > +4 is high.

On admission, P1NP = -0.80 ± 1.80 (S.D.), FDPD = 4.27 ± 2.32, NTX = 3.60 ± 2.06, CTx = 7.25 ± 3.09. On refeeding, P1NP = 4.97 ± 4.82*, FDPD = 4.23 ± 1.93, NTX = 2.19 ± 1.17, CTx = 4.01 ± 1.20*. On admission, BRBI (P1NP − FDPD) = -5.07 ± 2.72, (P1NP − NTX) = -4.40 ± 2.98, (P1NP − CTx) = -8.04 ± 4.26 and BTI (P1NP + FDPD) = 3.47 ± 3.13, (P1NP + NTX) = 2.80 ± 2.47, (P1NP + CTx) = 6.45 ± 2.73. On refeeding the BRBI (P1NP − FDPD) = 0.75 ± 4.76*, (P1NP − NTX) = 2.84 ± 4.22*, (P1NP − CTx) = 0.96 ± 4.33* and BTI (P1NP + FDPD) = 9.20 ± 5.60*, (P1NP + NTX) = 7.16 ± 5.61*, (P1NP + CTx) = 8.98 ± 5.54.

Formation (P1NP) significantly increased, while resorption (FDPD and NTX) remained high but CTX decreased after re-feeding. Any combination of BRBI and BTI illustrates severe negative remodelling and high turnover pre-feeding, and significantly positive remodelling and higher turnover post-feeding.

The BRBI demonstrated the uncoupling of resorption and formation and the significant improvement after refeeding. The importance of concurrent measurement of formation and resorption markers and the utility of the indices in understanding bone changes in AN were demonstrated.

TP2.06 SELECTED FOR POSTER CLINIC

DEVELOPMENT OF AN ID-LC-MS-MS METHOD FOR QUANTITATION OF SERUM 25-HYDROXYVITAMIN D2 AND D3

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Vitamin D is essential for maintaining calcium homeostasis, and its importance in paracrine functions is being elucidated. Vitamin D status is best assessed by the measurement of the 25 hydroxymetabolites (25-OH-D) in serum of the endogenously produced vitamin D3 and the exogenous D2 of plant origin. Both vitamin D2 and D3 can be supplemented in food and administered as pharmaceutical/OTC agents.

We report the development of an isotope dilution LC-tandem mass spectrometry (ID-LC-MSMS) assay for the simultaneous measurement of 25-hydroxyvitamin D2 and D3 in serum. A stable isotope Internal Standard (IS) (2H6-25-hydroxyvitaminD3 (26,26,26,27,27,27)) was used. Following optimisation, the system was operated in multiple reaction monitoring mode (25-hydroxyvitamin D2 $m/z+$ 413.5→395.4, 25-hydroxyvitamin D3 $m/z+$ 401.8 →383.5 and IS $m/z+$ 407.2 →389.4). Sample preparation involved precipitation of serum proteins (80:20 methanol:propanol containing IS), extraction into hexane, evaporation and reconstitution in the eluant. An 8 min gradient programme was run using methanol/formic acid (0.05%) on a C8 BDS column. RTs were 5.90 min (IS), 5.93 min (25-OH-D2) and 5.95 min (25-OH-D3). Quantitation was achieved by peak area ratios with reference to methanolic standards of 25-OH D2 and D3. The detection limit of the assay was better than 4 nmol/L (S/N ratio>10) for both analytes which equates to approximately 50 fmol detected by the instrument. Recoveries of added metabolites to 5 patient samples (range 9–83 nmol/l) ranged from 91–117% for 25-OH D3 and 94–108% for 25-OH D2. Interassay CV%s were less than 9% for 25-OH D2 and less than 5.5% for 25-OH D3.

We conclude that the use of ID-LC-MSMS provides a very sensitive, specific and simple system to quantify the 25-hydroxymetabolites of vitamin D in serum.

TP2.07

MORE THAN A SPLASH OF MILK WITH YOUR TB MAY BE TOO MUCH OF A GOOD THING

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The incidence of hypercalcaemia in granulomatous disease is low in the UK with <3% of patients hypercalcaemic at diagnosis. We describe two cases of pulmonary TB who were normocalcaemic at presentation, had a good clinical response to standard TB therapy and then several weeks later unexpectedly presented with hypercalcaemia.

Case 1 had been responding well to 7 weeks of DOT therapy for pulmonary TB when a routine blood sample showed his adjusted calcium to be 3.52 mmol/l. Although the patient was asymptomatic, renal ultrasound was consistent with nephrocalcinosis. He was treated with 4 l saline/furosemide and pamidronate 24 h later. Calcium levels quickly normalised and he remains normocalcaemic.

Case 2 had a good clinical response to 10 weeks of treatment for pulmonary TB when he presented feeling unwell. His adjusted calcium was 3.76 mmol/l but rapidly normalised after 24 hrs saline/furosemide, however 6 weeks later he was readmitted through A/E acutely unwell with an adjusted calcium of 4.01 mmol/l. Again calcium rapidly returned to normal after saline/frusemide and pamidronate. Subsequently he has remained normocalcaemic.

In both cases all other causes of hypercalcaemia were excluded including adrenal failure, thyrotoxicosis and occult malignancy. PTH and 25hydroxy vitamin D levels were appropriately suppressed.

It is not clear why these patients developed hypercalcaemia after weeks of treatment; however it is noteworthy that they both reported consumption of 2 pints of milk per day. It is likely that the high calcium load coupled to humoral production of 1, 25 Vitamin D precipitated their hypercalcaemia.

Monitoring of calcium levels during treatment of granulomatous disease is recommended.

TP2.08

A SIMPLE METHOD FOR MEASUREMENT OF VITAMIN D BINDING PROTEIN CAPACITY (DBPc) IN SERUM

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Vitamin D binding protein or Gc Globulin (DBP) is the major carrier of 25 hydroxyvitamin D (25D) and 1,25 dihydroxyvitamin D (1,25D) in blood. Methods that measure DBP in terms of mass do not necessarily indicate the functionality of this protein for carrying vitamin D compounds.

We have developed reagents that enable the functional binding capacity of DBP to be measured in serum.

Saturation of DBP was achieved by adding excess 25D (500 μL of 1200 nM) to 20 μL sample at 37 °C for 30 min. After 10 min chilling on crushed ice, cold charcoal reagent (500 μL) was added to the mixture and incubated for 1 h on the ice to remove unbound or low affinity albumin bound 25D. Following centrifugation of the charcoal, high affinity DBP bound 25D remains in the supernatant and was measured using the IDS Gamma B 25hydroxyvitamin D RIA kit.

The assay has a range of 1–10 μM DBPc. Sensitivity measured by 20 replicates of BSA Buffer plus 2SD was 0.87 μM. Intra-assay precisions on 20 replicates of 3 samples were 6.9% (1.5 μM), 8.2% (5.2 μM) and 6.3% (4.9 μM). Inter-assay precisions ($n=32$) from the same samples were 14.3% (1.5 µM), 10.2% (4.7 µM) and 7.8% (4.5 µM). The mean linearity of 5 samples diluted in BSA Buffer was 106% (1/2 dilution 108%, 1/4 dilution 105%). The mean value of 28 normal serum samples was 5.4 µM (range 2.4–8.6 µM).

The overall assay is completed in less than 5 h and can be used to obtain 25D and DBPc levels from the same assay. DBPc measures could also be used with a total 1,25D measure to calculate the relative amounts of 'free' 1,25D.

TP2.09

ROUTINE EXPERIENCE OF 25 HYDROXYVITAMIN D2 AND D3 ANALYSIS BY ISOTOPE DILUTION LC-TANDEM MASS SPECTROMETRY

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The 25-hydroxy-metabolites of vitamin D (D2 and D3) have generally been measured by competitive binding assays either employing antibodies or vitamin D binding protein and radioactive or non-isotopic labelled derivatives of vitamin D3. Most of these assays claim to measure both molecules equi-molarly, but there is evidence to the contrary. Kit details indicate they cross-react with the di-hydroxymetabolites.

In July 2003 we introduced an isotope dilution LC-tandem mass spectrometry (ID-LC-MSMS) method for the quantitation of 25-hydroxymetabolites of vitamin D2 and D3 into routine use. During this time we have analysed more than 5000 samples from a multiethnic community. Comparison of the total 25-hydoxyvitamin D (D2+D3) results obtained by ID-LC-MSMS with those obtained by radioimmunoassay (Diasorin), gave a regression line of $y=0.87x+0.9$, $r=0.93$ ($n=176$), but when results from those patients with a predominance (>60%) of 25-OH-D2 are correlated the slope is reduced to $y=0.84x+5.1$, $r=0.97$ ($n=19$). In our population the presence of predominantly 25-OH-D2 was commoner in groups from the Indian subcontinent.

The method is simple and has negated the need for radioactivity for the assay of vitamin D metabolites. We have found no co-eluting peaks with the same transitions as the metabolites or IS, however we have identified a bile acid precursor 7α-hydroxy-4-cholesten-3-one with the same transition as 25-Hydroxyvitamin D3 but with a different retention time.

We conclude that it is essential to measure both the 25-OH-D2 and D3 for monitoring vitamin D status and the ID-LC-MSMS has proved reliable and accurate and will be a useful tool for furthering the understanding of vitamin D metabolism.

TP2.10

HALF OF THE PREGNANT WOMEN FROM NON-EUROPEAN ETHNIC MINORITIES IN THE NETHERLANDS ARE SEVERELY VITAMIN D DEFICIENT

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Immigrants from non-European countries living in Western Europe have a high risk of developing vitamin D deficiency, defined as calcidiol <30 nmol/l. In vitamin D deficiency specific symptoms like fatigue, muscle pain and weakness are most common. These symptoms are also frequently observed in pregnant women. Consequently, a vitamin D deficiency in pregnancy is often not recognised and remains therefore untreated.

Aim: Determination of the prevalence of vitamin D deficiency among pregnant women from non-European origin compared to Dutch/ European pregnant women.

Patients and methods: Calcidiol and calcium were determined in the 10th or 30th week of pregnancy in subsequent women of Dutch/ European and non-European origin visiting the Obstetrics Department in a regional hospital (Amersfoort, the Netherlands). From April to December 2004 we included 96 mothers from non-European ethnic minorities (Moroccan, Turkish, African and Asian) and 403 Dutch/European mothers.

Results: In the ethnic non-European group 71% were vitamin D deficient (calcidiol<30 nmol/l) and 49% were severely deficient (calcidiol<20 nmol/l). In the Dutch/European group only 8% were vitamin D deficient and 2% severely deficient. The calcium levels in both groups did not differ significantly.

Conclusion and discussion: Vitamin D deficiency is very common among pregnant women from non-European ethnic minorities in Amersfoort and probably in the rest of the Netherlands. Since vitamin D deficiency mimics usual weakness and fatigue in pregnancy it remains unnoticed, preventing the beneficial supplementation both for the mother and her unborn child.

TP2.11

AUTOMATION OF A 25-HYDROXYVITAMIN D EIA ON THE GRIFOLS TRITURUS

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As specialist tests become more widely requested in the clinical laboratory, the need for automation of labour intensive manual methods increases. An example is 25-hydroxyvitamin D which has become a routine test. IDS manufactures a 25-Hydroxyvitamin D kit which is marketed worldwide and is a manual 96-well EIA.

Using the Grifols Triturus open automated immunoassay system, IDS has developed a protocol for automation of the OCTEIA 25-Hydroxyvitamin D kit (25(OH)VitD), which meets the performance criteria set for the manual method. The Triturus method procedure differs from the manual method to optimise instrument settings. Incubation times and reagent volumes are identical in both methods.

A total of 132 normal serum and plasma samples appropriate for the working range were assayed in both the manual and Triturus methods. These were tested across five manual assay settings and five Triturus settings. Correlation of the Triturus method using Passing-Bablok was 1.02×manual 25(OH)VitD+1.8 nmol/L with R^2 of 0.945 ($r=0.972$).

No significant bias was identified; Triturus −3.8% versus manual. Sensitivity was determined as 4.9 nmol/L (1.96 ng/ml) which is within the specification of <6 nmol/L. Intra-assay precision assessed by measurement of multiple replicates of three serum controls was 3.7% (48.3 nmol/L), 3.0% (89.6 nmol/L) and 5.3% (223 nmol/L). Inter-assay precision was calculated from the coefficient of variation of the same controls from 21 separate assays and was 6.0% (48.6 nmol/L), 5.9% (85.8 nmol/L) and 10.1% (217 nmol/L). Both fall within the specification set for the manual method.

A fully automated protocol for IDS 25-Hydroxyvitamin D EIA on the Grifols Triturus has been developed which shows equivalence to the manual method.

TP2.12

OSTEOPROTEGERIN LEVEL IN OLDER WOMEN WITH FIRST OSTEOPOROTIC FRACTURE

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Objective: The objective of this preliminary study was to assess the serum levels of osteoprotegerin (OPG), osteocalcin (OC) and crossLaps (CTx) in patients with first osteoporotic fracture.
Methods: Eighteen patients (women) aged 71–89 years were included in the study. Patients were divided in two age groups: A: 70–79 (7 women) and B: 80–89 years (11 women). Fasting blood was taken for laboratory analysis the next day after osteoporotic fracture. Serum osteoprotegerin was determined by ELISA (Biomedica) and bone turnover markers: osteocalcin and CTx by immunoassays (Brahms Ag and Roche, respectively).
Results: Levels of osteoprotegerin (202.6 ± 76 vs. 180 ± 50 pg/ ml), CTx (0.52 ± 0.22 vs. 0.426 ± 0.215 ng/ ml) and osteocalcin (6.3 ± 4.6 vs. 4.73 ± 2.1 ng/ ml) were not significantly different between groups B and A. However, in group A mean OPG value was much higher than in age-adjusted controls and a tendency was observed to increased OPG values with age. In group B OPG level was within the reference range, a strong negative relationship was found between bone turnover markers: OC and CTx ($r = -0.73$; $p < 0.039$) and CTx level correlated significantly with age ($r = 0.65$; $p < 0.03$).
Conclusions: Higher circulating OPG levels in older women with first osteoporotic fracture may reflect a compensatory mechanism serving to limit enhanced bone loss in elderly.

TP2.13

BONE RESORPTION IN HYPERTHYROID PREMENOPAUSAL WOMEN ON THYROSUPPRESSANT THERAPY

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Objective: The aim of the study was to investigate the dynamics of bone resorption markers (blood β-crosslaps, urinary pyridinoline, deoxypyridinoline and hydroxyproline) in hyperthyroid premenopausal women treated with thiamazole.
Patients and methods: The study included 45 premenopausal hyperthyroid women with no other cause of increased bone turnover. An informed consent was obtained from all study subjects. Thyroid hormones, TSH and bone resorption markers were determined at baseline, and then at 6 and 18 weeks of treatment. Statistical analysis was performed using Wilcoxon matched pair analysis.
Results: Thyrosuppressant therapy proved successful. At 6 weeks, elevated T3 and T4 values were measured in only four women, and all women had TSH values below or at the upper reference limit. At 6 weeks, a statistically significant decrease was recorded in blood β-crosslaps, urinary pyridinoline and deoxypyridinoline and urinary hydroxyproline concentrations ($p < 0.01$ all). At 18 weeks of therapy, all study parameters showed a decrease compared to their 6-week concentrations, however, only the decrease in the blood β-crosslap concentration was statistically significant ($p < 0.05$).
Conclusion: Although the rate of bone resorption was considerably slowed down by thyrosuppressant therapy, the medians of some markers were still elevated in week 18 (pyridinoline, deoxypyridinole, blood β-crosslaps), indicating an increased rate of bone resorption in our patients. Sequential determination of any of these markers may be used to determine bone resorption in hyperthyroid patients.

TP2.14

INAPPROPRIATE PTH RESPONSE TO HYPOCALCAEMIA IN HIV INFECTED PEOPLE–AN OBSERVATIONAL STUDY

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Abnormalities in bone metabolism have been observed in HIV+ patients both on and off antiretroviral therapy. Deficient parathyroid hormone responses and/or low levels of vitamin D metabolites have been demonstrated in HIV+ patients. We describe our observations in a group of HIV+ patients with low serum calcium levels.
22 patients with HIV and 12 controls with borderline low serum calcium had PTH levels measured. 19 patients with HIV were on antiretroviral therapy. Serum levels of calcium were measured using an Olympus AU 640 and PTH assayed using an Immulite 2000. Differences in the medians were compared using the nonparametric Mann–Whitney test.
Mean serum calcium levels in HIV+ patients were not significantly different from the control group ($p > 0.05$). In the HIV+ group (with the exception of three patients who had only modest increases in PTH) the PTH levels were inappropriately low. The control group median for PTH was 28.3 and that of the HIV+ group was 3.95 pmol/L ($p < 0.0016$).
The main finding in this group of patients is that of inappropriately low PTH levels. The mechanism of this "acquired" hypoparathyroidism is unknown although it is interesting that the bone pathology in patients with HIV is similar to hypoparathyrodism.

TP2.15

NUTRITIONAL FACTORS INFLUENCING OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

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Introduction: Prevalence and incidence of osteoporosis in Slovakia are increasing. A lot of lectures and articles were prepared with the aim of informing people about the appropriate diet. We show some results at the present time.

Material and methods: 83 postmenopausal women (age 60 ± 9.7 years) with osteoporosis (BMD<2.5 S.D.s of young normal population) were included in the study. Patients were in a steady state and had no kidney, gastrointestinal or endocrine system pathology. Albumin, calcium, magnesium, creatinine and urea were analysed on Integra 800 (Roche, Switzerland), beta-cross laps (CTx) and osteocalcin on Elecsys 2010 (Roche, Switzerland).
Results: Protein and mineral intake was in general low in our group of patients. We found 22% of women with calcium deficit, 10% had protein malnutrition (albumin<38 g/l). Median of 24 h urine urea excretion was 306 mmol/24 h; 42% of women had urea excretion below 300 mmol/24 h. Negative correlation was found between age and serum albumin concentration as well as 24 h urine urea excretion ($p<0.02$ for both). Older women had higher fractional Mg excretion ($p<0{,}001$). 63% of women were overweight (BMI>25); in 19% was BMI<22, but it did not significantly influence any of the other analysed parameters. 24 h urea excretion correlated significantly with calcium and magnesium excretion ($p<0.001$, $p<0.02$, respectively). Patients with lower albumin concentration and magnesium excretion had higher CTx concentration, but changes were not significant ($p<0.1$ for both).
Conclusions: We confirmed in our study the important role of protein and mineral intake for osteoporosis development in postmenopausal women. Elderly women had lower protein and mineral intake. BMI did not influence any of the studied parameters.

TP2.16

RANDOM CALCIUM/CREATININE RATIO VS. 24-H URINARY CALCIUM OUTPUT IN THE ASSESSMENT OF PATIENTS WITH METABOLIC BONE DISEASE (MBD)

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MBD is often caused by imbalance in vitamin D, calcium or phosphate homoeostasis. Management may require patients to receive calcium and/or vitamin D supplements.
Aim: To investigate the correlation between random urine (Ca/Cr ratio) and 24-h urinary calcium output (24-hrUCa) and their relation to MBD and markers reflecting calcium homeostasis.
Results: Between Jan 2002 and July 2004, 12,604 urinary samples were analysed. A significant correlation ($r=0.747$, $P<0.0001$) with an Odds ratio of 5.26 (95 CI, 5.15–5.37) existed between Ca/Cr ratio and 24-hrUCa. Cross tabulations with 24-h urinary creatinine output showed the majority of the discordance was explained by inappropriate urine collection.
Relationship to MBD revealed some disparity, 24-hrUCa was high in subjects with celiac disease and liver cirrhosis while Ca/Cr ratio was low.
Females had significantly higher Ca/Cr ratio compared to males (0.56 ± 031 vs. 0.40 ± 0.22), a significant trend with advancing age only seen in females. However, 24-hrUCa was similar for both genders (Female 3.67 ± 2.0 vs. Male 3.75 ± 2.32) with no trend with age.
PTH positively correlated with CTX ($r=0.4$, $P<0.0001$), ALP ($r=0.24$, $P<0.0001$), negatively correlated with 24-hrUCa ($r=-0.2$, $P<0.0001$), Ca/Cr ratio ($r=-0.1$, $P<0.0001$), PO4 ($r=-0.18$, $P<0.0001$) and total vitamin-D ($r=0.23$, $P<0.0001$).
Conclusion: Ca/Cr ratio increased with advancing age highlighting the role of the renal tubules in the negative calcium balance of women with osteoporosis. Ca/Cr ratio permitted an easy, rapid and inexpensive estimation of the daily urinary calcium excretion and was also a marker of renal tubular calcium handling.

TP2.17

DEVELOPMENT AND EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF INTACT PTH ON THE ABBOTT AxSYM® ANALYZER

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Measurement of Intact Parathyroid Hormone (IPTH) has clinical significance in the differential diagnosis of hypercalcemia and hypocalcemia. We report on results from the development of a highly specific automated assay for IPTH on the Abbott AxSYM analyzer.
The assay principle is based on microparticle enzyme immunoassay (MEIA) technology. IPTH present in a specimen is selectively bound by anti-IPTH antibody-coated microparticles forming an antigen–antibody complex. After initial incubation, the antigen–antibody complex is transferred to a glass fiber matrix cell where unbound material is washed from the matrix cell. Biotinylated anti-IPTH antibody is then added to the matrix cell and incubated. After a further wash step, detection is achieved through an anti-biotin alkaline phosphatase conjugate and a fluorogenic enzyme substrate, the fluorescence intensity is measured by the AxSYM optical assembly.
Data from total precision studies following the NCCLS guidelines over 20 days (2 runs/day 2 replicates/run) at concentrations of 37.4, 194.9 and 648.2 pg/mL were 8.6, 8.5 and 8.1% CV, respectively. The mean analytical sensitivity, assessed with one reagent lot, 2 different AxSYM instruments, was <3 pg/mL.
A preliminary correlation with a commercially available assay for IPTH resulted in $r>0.9$. The time to first result for the AxSYM assay is 20 min with throughput of 42 tests per hour. Based on our evaluation, we conclude that the AxSYM IPTH assay is a sensitive, rapid, precise and accurate method for measuring Intact PTH levels in human serum or plasma.

TP2.18

THE INCREASE IN CIRCULATING FIBROBLAST GROWTH FACTOR 23 AFTER PHOSPHATE INGESTION IN HUMANS

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We measured fibroblast growth factor 23 [FGF-23] with an ELISA plate assay [human FGF-23 [c-term], Immutopics Inc, Ca, USA] in the serum of 6 subjects [age 45 ± 4 yr,M4, F2] between 2000 and 0600 h. The measurements were made on samples

prior to, and after ingestion at 2000 h of 750 mg of phosphate [Sandoz] equivalent to a phosphate loading in a large meal. Mean baseline FGF-23 was 109 ± 61 RU/L [range 56–220] which increased to 202 ± 156 RU/L [range 59–493] after 30 min, an average increase of 71% and reached a maximum of 395 ± 330 RU/L [range 104–1038] on average 2 h post phosphate ingestion the mean increase was 248 ± 149% [$p = 0.02$]. In 4/6 subjects [67%] FGF-23 returned to pre-phosphate values after 3.5 h [means 84 vs. 79 RU/L] whilst the remaining 2 subjects, with higher baseline concentrations, took longer to return to baseline. All had returned to baseline within 7 h from phosphate ingestion. The ingestion of 750 mg of phosphate at 2200 h induces a rapid rise in circulating FGF-23 that reaches a peak approximately 2 h post ingestion. The increase in circulating FGF-23 we observed is likely to be seen following food, suggesting that fasting samples should be used when evaluating serum FGF-23 concentrations in pathological diseases.

TP2.19

INCREASE IN SERUM OSTEOCALCIN LEVELS WITH HIGH DOSE STATIN TREATMENT IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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Statins have been suggested as potential therapeutic agents in osteoporosis and are associated with reduced fracture risk. The aim of this study was to investigate the effects of high and low dose statin treatment on biochemical bone markers in patients with Familial Hypercholesterolaemia (FH) and Non-Familial Hypercholesterolaemia (NFH).

30 FH patients (10 males, 20 females, mean ± S.D. age = 44.5 ± 10.3 years) and 30 NFH (15 males, 15 females, mean ± S.D. age = 49.8 ± 8.8 years) were recruited. The NFH and FH patients were treated with atorvastatin 10 and 80 mg/day, respectively. Serum levels of osteocalcin (OC), carboxy-terminal fragment of type I collagen (CTX), fasting serum lipids, calcium, phosphate, total alkaline phosphatase (ALP) and parathyroid hormone (PTH) were measured at baseline and 6 months after treatment.

At baseline, there were no differences in OC and CTX levels in FH and NFH patients. In the FH patients, there was significant increase in OC levels at 6 months compared to baseline (mean ± S.E.M. = 34.4 ± 2.3 vs. 24.8 ± 2.0 ng/ml, $p < 0.05$) but in NFH patients there were no difference in OC levels before and after treatment (mean ± S.E.M. = 29.3 ± 1.3 vs. 28.3 ± 0.9 ng/ml). There were no differences in CTX levels at 6 months compared to baseline, in both FH (median [95%CI]: 0.58 [0.10–0.66] vs. 0.59 [0.57–0.64] ng/ml) and NFH (median [95%CI]: 0.59 [0.24–0.62] vs. 0.58 [0.53–0.64] ng/ml) groups.

High dose statin treatment in FH, but not low dose statin in NFH is associated with improvement in bone formation. This suggests a beneficial potential role of high dose statin in improving bone formation.

TP2.20

EVALUATING BONE METABOLISM IN PATIENTS WITH MONOCLONAL GAMMOPATHY

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Multiple myeloma (MM) is characterised by unique bone formation/resorption leading to osteolysis. Skeletal involvement is frequent and its occurrence increases with the progression of the disease. We monitored levels of bone specific markers in patients with MM ($n = 12$), with Monoclonal Gammopathy of Undetermined Significance (MGUS) ($n = 13$) and healthy controls ($n = 14$) over 2 years.

We measured the levels of serum alkaline phosphatase (ALP), bone specific alkaline phosphatase (BAP), osteocalcin (OC), calcium (Ca), phosphate (P), CRP, and urine deoxypiridinoline (DPD), calcium (Ca-U), phosphate (P-U) and creatinine (Crea-U). BAP was determined by elctrophoresis using Sebia system. Ca, P, creatinine and ALP were determined using traditional chemical methods. OC and DPD were measured using immunoassays and CRP using immuno-turbidimetry.

In the MM group the level of DPD increased significantly ($p < 0.07$) and the level of Ca-U decreased significantly comparing the 2004 results to 2003. Other results were unchanged. In the MGUS group DPD levels increased significantly ($p < 0.04$) in 2004.

ALP levels were significantly lower in both years in the MM group than in the MGUS group ($p < 0.07$ in 2003, $p < 0.09$ in 2004). BAP levels were significantly lower both in the MGUS group ($p < 0.02$ in 2003, $p < 0.004$ in 2004) and in the healthy controls ($p < 0.04$ in 2003, $p < 0.05$ in 2004). OC levels were significantly higher in 2003 in the MGUS group than in the MM group ($p < 0.02$) or the healthy controls ($p < 0.008$).

We conclude that bone markers reflect the aspects of bone disorders well, hence determining them gives valuable information about the progression of the disease in MM patients. Their combined use is valuable in the treatment of MM patients. We found monitoring BAP and DPD most useful.

TP2.21

THREE MONTH BONE MARKER RESPONSE TO TERIPARATIDE TREATMENT IN AN ELDERLY OSTEOPOROTIC POPULATION

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Teriparatide is a recombinant form of the 34 amino-terminal amino acids of human parathyroid hormone (PTH). It is the first agent to stimulate new bone formation and has been shown to improve bone microarchitecture, increase bone mass, and prevent vertebral and nonvertebral fractures.

Our objective was to investigate the response of biochemical markers of bone turnover to 3 months of treatment with teriparatide in an elderly population. Thirty-two patients (64–90 yrs) with established osteoporosis, previous fracture, and inadequate response to other

treatments were recruited. Baseline fasting bloods were taken. Patients self-administered 20 μg of teriparatide by subcutaneous injection daily for 3 months and fasting bloods were again taken. Serum markers assessed were C-Telopeptide (CTX) (a collagen derived marker of bone resorption), Osteocalcin (a protein marker of bone formation), and P1NP (a collagen derived marker of bone formation). Baseline and post-treatment PTH was also measured.
3 months treatment with teriparatide yielded significant changes in the rate of bone turnover. Mean values for the formation markers osteocalcin and P1NP increased 310% and 346%, respectively, compared to baseline. The resorption marker CTX increased by 246%. Mean baseline PTH concentration was 31.2 pg/ml and at 3 months was 22.0 pg/ml.
Bone markers are valuable tools for assessing early response to teriparatide treatment. The results show that the percentage rise in formation markers is greater than that of the resorption markers indicating net bone formation is occurring as early as 3 months post treatment. The results are also indicative of patient compliance with the treatment.

TP2.22

OSTEOPROTEGERIN AND RECEPTOR ACTIVATOR OF NF-kappaB LIGAND IN SYNOVIAL FLUID AND SERUM IN PATIENTS WITH SEVERE PRIMARY KNEE OSTEOARTHRITIS

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In this study we evaluated the relationship between osteoprotegerin (OPG), a recently identified cytokine that acts as a decoy receptor for the receptor activator of NF-kappaB ligand (RANKL), in synovial fluid and serum of 25 patients (17F/8M, mean age 53 y) with severe primary knee osteoarthritis (OA) and disease severity graded radiologically. Synovial fluid was aspirated from the affected joint during surgery, where a total knee arthroplasty was performed. Blood samples were obtained from the same patients 1–2 h before surgery. Serum and synovial fluid concentrations of OPG and RANKL were determined by immunoenzymatic assays. We found that OPG concentration in synovial fluid was significantly higher than in serum (257.5 ± 76.9 vs. 49.2 ± 10.1 pmol/l, $p < 0.00001$), indicating that the increase of OPG in patients with knee OA is not systemic, but rather localized in the affected joint. OPG levels were increased in synovial fluid in relation with the severity of knee OA and were significantly higher in patients with grade 4 than in those with grade 0 or 1 OA. RANKL levels were found to be low in both synovial fluid and serum 1.5 ± 0.4 and 1.0 ± 1.0 pmol/l, respectively). Serum levels of OPG and RANKL did not correlate with the severity of knee OA. These data suggest that OPG plays a protective role in avoiding further cartilage destruction in knee OA or may reflect a compensatory response by chondrocytes or synovial fibroblasts in destabilization of the normal coupling of degradation and synthesis of articular cartilage.

TP2.23

VALUES OF OSTEOPROTEGERIN AND RANKL BY PATIENTS WITH BREAST CANCER AND BONE METASTASES BEFORE AND AFTER THERAPY WITH BISPHOSPHONATES

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Receptor activator of nuclear factor-κB ligand (RANKL), a member of the family of tumor necrosis factor is essential for osteoclast formation and activation. Osteoprotegerin (OPG) is a soluble decoy receptor that inhibits osteoclast differentiation through its binding to RANKL.
The aim of recent study is to assess the change in serum concentration of OPG and RANKL before and after therapy with bisphosphonates by patients with advanced breast cancer and bone metastases.
Twenty female patients with breast cancer and established bone metastases were investigated before and after 3 months treatment with bisphosphonates (monthly infusion), compared to 20 healthy nonmenopausal women (30–40 years old). Serum levels of OPG and RANKL were determined using sandwich ELISA methods (BIOMEDICA, Austria).
No significant elevation was observed in serum concentration of OPG and RANKL in cancer patients versus healthy women, and only a tendency for higher concentration by patients with metastatic bone disease (OPG mean value in pretreatment group 3.66 ± 1.06; in posttreatment group 3.98 ± 1.22; in control group 3.6 ± 0.7 pmol/l; RANKL mean value in pretreatment group 3.21 ± 2.7; in post-treatment group 2.83 ± 1.39; in control group 2.43 ± 1.58 pmol/l). Because of the large inter-individual differences no definitive conclusion regarding changes due to therapy was possible.
Thus, the measurement of OPG and RANKL levels and their ratio may have potential value in the assessment of metastatic bone disease. Further extended studies are needed to clarify this hypothesis.

TP2.24

BONE TURNOVER MARKERS IN EARLY DETECTION OF BONE METABOLISM AND DISEASES

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Metabolic bone diseases are considered to be a major health problem, particularly in the elderly. In this study the level of osteocalcin and C-terminal propeptide of type I collagen (CICP) in sera (bone formation markers) and deoxypyridinoline (Dpd) (bone resorption markers) in urine were investigated. All biochemical markers were determined by monoclonal competitive enzyme immunoassay (EIA) obtained by Metra Bio systems (Oxford, UK). The obtained mean values in the control group ($n = 45$) of healthy post-menopausal women were for osteocalcin 16.36 ± 5.85

ng/mL, for CICP 157 ± 16.83 ng/mL and for Dpd 9.01 ± 3.29 nmol Dpd/mmol creatinine. The mean values for the postmenopausal osteoporosis group were for osteocalcin 39.62 ± 13.45 ng/mL, CICP 231.87 ± 6.76 ng/mL and Dpd 13.22 ± 3.38 nmol Dpd/mmol creatinine. The mean values for the hyperthyroid group for osteocalcin were 24.00 ± 8.1 ng/mL, CICP 186.15 ± 22.87 ng/mL and Dpd 10.59 ± 2.79 nmol Dpd/mmol creatinine. The mean values for the hyperparathyroidism group for osteocalcin were 45.20 ± 14.83 ng/mL, CICP 277.57 ± 89.93 ng/mL and Dpd 13.11 ± 4.43 nmol Dpd/mmol creatinine. In the malignancy group the mean values for osteocalcin were 23.92 ± 7.55 ng/mL, CICP 176.64 ± 11.44 ng/mL and Dpd 9.76 ± 2.65 nmol Dpd/mmol creatinine. These bone markers are non-invasive and allow a dynamic insight into bone metabolism.

TP2.25

BEHAVIOUR OF SERUM PROCOLLAGEN TYPE 1 AMINO-TERMINAL PROPEPTIDE (S-P1NP) IN COMPARISON WITH OTHER BONE TURNOVER MARKERS

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(i) To compare the reference limits of the bone markers in an Estonian population sample with those recommended by the manufacturers of the reagents.
(ii) To assess the analytical and biological variation of S-P1NP.
(iii) To examine the behaviour of S-P1NP among bone turnover markers.

Twenty-three healthy women, aged 31–48 (mean 37), with a normal lumbar DXA finding served as the controls. The study group consisted of 17 patients with postmenopausal osteoporosis (48–65, mean 56 years). Eight of them had received risedronate therapy for 6 months. Bone resorption was assessed by collagen type I C-terminal telopeptide in serum (S-CTx-I) and by urinary free desoxypyridinoline (U-fDpd). Bone formation was assessed by S-P1NP and osteocalcin (S-Oc) levels.

Comparison of the upper limits of the normal (ULN) levels in the controls and the respective levels recommended by the manufacturers demonstrated that the ULN values were 48% lower for S-CTx-I and 14% lower for S-Oc, 35% higher for U-fDpd/creatinine and 22% higher for S-P1NP than the respective values given by manufacturers. Analytical variation of S-P1NP was 1% ($n=11$), biological 11% ($n=10$). Before risedronate therapy, the two formation markers revealed a significant correlation (rho=0.574, $p=0.02$). A surprisingly strong correlation was found between S-P1NP and S-CTx-1 before (rho=0.541, $p=0.03$) and during therapy (0.726, $p=0.001$). The regression models including S-Oc or S-CTx-1 allow us to predict 25% or more of the variability of S-P1NP.

Conclusions.

S-P1NP is an analytically precise and stable test.

An appreciable proportion of S-P1NP variability is predictable by S-Oc and S-CTx (one resorption marker) but not by U-fDpd.

TP2.26

CHANGES OF BONE METABOLISM DURING PUBERTY

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Adolescence is the period during which the greatest accrual of bone mineral occurs. After puberty, biochemical markers of bone turnover remain slightly elevated compared to levels in the mature adult.

Our aim was to establish the reference values in 175 (mean age: 17 ± 1 years) secondary school students (101 girls, 74 boys). Physical activity, dietary calcium intake and body mass index (BMI) were measured. Bone mineral density (BMD), *Z*-score (DEXA) and serum biochemical markers (osteocalcin; OC and beta-crosslaps; βCL) were measured (ECLIA, Elecsys 2010, Roche).

Results: significant negative correlation was observed between *Z*-score and OC ($r=-0,43$); and between BMD and βCL ($r=-0,34$) values. In 14 teenagers lower bone mineral density was observed, all of them have very low Ca2+ intake (less than 850 ± 281 mg per day). They have significantly higher OC levels (139 ± 75 ng/mL) than the ones with normal *Z*-score (75 ± 43 ng/mL). In boys significantly higher βCL (1.5 ± 0.7 ng/mL) and OC (103 ± 46 ng/mL) levels were measured compared to girls (βCL: 0.8 ± 0.4 ng/mL; OC: 55 ± 26 ng/mL). Significantly higher OC levels (99 ± 51 ng/mL) were measured in the thin (BMI<19) youngsters, than in the group with normal body mass index (BMI: 19–25; OC: 73 ± 39 ng/mL), or in overweight adolescents (BMI>30; OC: 44 ± 16 ng/mL).

Conclusions: the results draw attention to malnutrition among teenagers. In establishing appropriate reference ranges, it is advisable that boys and girls are analysed separately and the BMI values are considered.

TP2.27

A RARE CASE OF METABOLIC BONE DISEASE: POLYOSTOTIC FIBROUS DYSPLASIA AND GROSS HYPERPARATHYROIDISM

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A 26 year old Chinese female presented to A and E with a non-traumatic fracture of the left humerus. She had previously undergone pelvic surgery for an unspecified bone disease.

Serum results on admission were as follows: adjusted calcium 3.28 mmol/L, phosphate 0.48 mmol/L and alkaline phosphatase 516 U/L, parathyroid hormone 123 pmol/L, 25 OH vitamin D3<15 nmol/L and 1.25 OH vitamin D3 120 pmol/L. Apart from the fracture, X-rays showed osteolytic lesions in the left humerus, both proximal femora and pelvis. The fracture was surgically stabilised. Core needle biopsy of the humerus was taken as the differential diagnosis included primary or secondary bone cancer.

Histology of the humeral biopsy was reported to be consistent with a brown tumour of hyperparathyroidism. Sestamibi scan

confirmed the presence of a parathyroid adenoma. A right-sided parathyroidectomy was carried out in November 2004. Post-operatively, the 'hungry bone syndrome' was observed, with an increased calcium requirement, and persistently raised alkaline phosphatase.

Isotope bone scan was reported to show features typical of fibrous dysplasia involving the skull, mandible, mid-shaft of right humerus, both iliac bones, left femoral head, upper third of right femoral shaft, proximal left tibia and distal right tibia. Skin pigmentations were not observed. A history of precocious puberty was not elicited.

Endocrinopathies are occasionally associated with polyostotic fibrous dysplasia, but the coexistence of hyperparathyroidism and fibrous dysplasia is rare. At this stage it is still unclear whether the multiple bony lesions are due to hyperparathyroidism or fibrous dysplasia. It is planned to repeat the isotope bone scan and X-rays after an appropriate period of calcium and vitamin D replacement.

TP2.28

A COMPARISON OF TELOMERE LENGTH MEASURED BY RT-PCR IN OSTEOPOROTIC AND NON OSTEOPOROTIC PATIENTS

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Accelerated aging, resulting in premature loss in proliferative capacity has been proposed as a pathogenic cause of reduced bone formation in osteoporotic patients.

The aim of this study was to compare the ageing process in osteoporotic and non-osteoporotic patients using telomere length measured in blood leukocytes as a marker of generalised cellular age.

Telomere length was determined by quantitative PCR in leukocyte DNA samples collected from 321 patients (266 female and 55 male) referred to Belfast City Hospital's osteoporosis clinic. BMD measurements at the lumber spine and hip and clinical history were available for each patient.

The quantitative PCR method for the determination of relative telomere length was linear over a DNA range of 0.9–35 μg/mL, with a precision of <12%. Relative telomere length in patients attending the osteoporosis clinic is weakly correlated with chronological age ($r^2=0.0724$; $p<0.01$). There was no significant difference in relative telomere length (mean+S.E.) [0.757+0.0226 vs. 0.719+0.0161, $p>0.05$] between osteoporotic and non-osteoporotic patients.

We conclude that relative telomere length determined by quantitative PCR was as good an indicator of cellular age as more traditional and labour intensive methods of telomere length determination. Our data comparing relative telomere length suggest that patients with osteoporosis do not show evidence of generalised accelerated cellular aging, relative to non-osteoporotic patients.

TP2.29

BIOCHEMICAL INDICES OF BONE METABOLISM ARE USEFUL IN ASSESSING METABOLIC BONE DISEASE IN PRIMARY HYPERPARATHYROIDISM

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Primary hyperparathyroidism often presents with mild clinical symptoms, however underlying skeletal involvement is common. The aim of this study was to apply indices of bone remodelling (bone remodelling balance index (BRBI), (bone formation − bone resorption)) and bone turnover (bone turnover index (BTI) (bone formation+bone resorption)) to assessing the impact of PTH on bone in patients with mild (PTH<141 ng/L, $n=14$) and severe (PTH>225 ng/L, $n=8$) primary hyperparathyroidism (reference range 2–52 ng/L).

Serum markers of bone formation (bone alkaline phosphatase (BAP), procollagen type 1 N-terminal propeptide (P1NP), intact osteocalcin (OC)) and urine markers of bone resorption (N-terminal telopeptide of type 1 collagen (NTX), deoxypyridinoline cross-links (DPD)) were measured. Each result was expressed as a T value ((observed result − mean of reference range)/S.D. of reference range), a normal T value being ± 2.

In mild hyperparathyroidism, the T values (mean $\pm$ S.D.) for BAP, OC and PINP were 2.52 ± 3.20, 1.65 ± 2.84 and 0.89 ± 2.33, respectively, and for NTX and DPD were 1.45 ± 2.40 and 3.86 ± 5.29, respectively. In severe disease, T values for BAP, OC and PINP were elevated at 5.59 ± 9.96, 10.37 ± 16.24 and 3.85 ± 8.68, respectively, as were NTX and DPD at 7.38 ± 10.38 and 4.75 ± 7.43, respectively. The mean BRBI (PINP − NTX) was -0.55 ± 1.91 in mild and -3.53 ± 2.89 in severe disease ($p<0.02$). The mean BTI (NTX+PINP) was 2.74 (range $-2.76 \rightarrow 11.22$) in mild and 11.278 (range $-3.26 \rightarrow 51.33$) in severe disease. These results demonstrate greater negative bone remodelling and higher bone turnover leading to bone loss in severe compared with mild hyperparathyroidism, helping our understanding and assessment of the pathophysiology of bone disease in primary hyperparathyroidism.

TP2.30

A NEW COMMERCIAL, NON-ISOTOPIC METHOD FOR THE MEASUREMENT OF 1,25-DIHYDROXYVITAMIN D IN HUMAN SERUM AND PLASMA

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The hormone 1,25-dihydroxyvitamin D (1,25D) produced by hydroxylation of 25-hydroxyvitamin D (25D) in the kidney is one of the major regulators of calcium metabolism and acts on a variety of target tissues.

Using the simple IDS immunoextraction method, we have developed an enzyme immunoassay (EIA) for the measurement of 1,25D in human serum and plasma.

Delipidated samples are incubated with a solid phase bound anti-1,25D monoclonal antibody. The purified 1,25D is eluted and dried. The dried eluate is reconstituted with sheep anti-1,25D polyclonal antibody, incubated and transferred to a microtitre plate coated with an anti-sheep antibody. Biotin labelled 1,25D is added, incubated and amount of bound biotinylated 1,25D determined by an Avidin labelled HRP/TMB colorimetric detection system. The endpoint colour developed for samples is inversely proportional to the amount of 1,25D present.

Performance for the IDS OCTEIA 1,25-Dihydroxy Vitamin D EIA was as follows. The assay has a range of 0 to 500 pM. Sensitivity was 6.3 pM. Correlation (linear-regression) with the IDS Gamma-B 1,25-Dihydroxy Vitamin D RIA was EIA = 0.996RIA − 0.65 pM, correlation coefficient (r value) of 0.960 (n = 167). Intra-assay precision (20 replicates, 3 samples) was 17.8% (17.6 pM), 10.8% (47.4 pM) and 9.0% (140.6 pM). Inter-assay precision (n = 25) from the same samples was 14.0% (18.1 pM), 15.6% (50.8 pM) and 13.8% (133.6 pM). Mean linearity of 5 samples diluted in assay buffer was 92%. Mean recovery based on 5 samples was 94%.

The IDS OCTEIA 1,25-Dihydroxy Vitamin D EIA demonstrates excellent correlation with the existing IDS Gamma-B 1,25-Dihydroxy Vitamin D RIA and being non-isotopic has numerous advantages over existing isotopic methods.

TP2.31

HORMONE REPLACEMENT THERAPY AND BONE RESORPTION IN POSTMENOPAUSAL WOMEN WITH RHEUMATOID ARTHRITIS

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Background: The serum CrossLaps assay recognizes C-terminal degradation products of type I collagen. Serum CrossLaps are a marker of bone resorption and has been shown to have a clinical value in the evaluation of the effect of anti-resorptive therapy in postmenopausal patients with osteoporosis.

Objective: To investigate the effects of hormone replacement therapy (HRT) on bone metabolism in postmenopausal women with rheumatoid arthritis (RA).

Methods: Patients with RA, 55 women (mean age 59 yr) were randomized to HRT (n = 30) or placebo (n = 25) in this double blind, placebo-controlled trial for 1 year. Outcome measures were hip and spine BMD, measured by dual energy X-ray absorptiometry and biochemical marker of bone resorption serum CrossLaps measured by the Roche Elecsys 2010 chemiluminescence immunoassay.

Results: Basic serum CrossLaps levels were significantly elevated in all patients compared to 35 year old female healthy subjects as controls (p < 0.01). Serum CrossLaps levels decreased significantly in RA patients treated with HRT but not in the placebo group. Six months changes in serum CrossLaps levels correlated with 1 year changes in spinal BMD (p < 0.01) in RA patients treated with HRT.

Conclusion: Serum CrossLaps can be used to measure bone resorption and to monitor the effect of HRT in patients with RA. Early changes in serum CrossLaps levels predict long-term changes in vertebral BMD in elderly women with RA receiving HRT.

TP2.32

SERUM INFLAMMATION MARKERS AND 25-HYDROXYVITAMIN-D IN RHEUMATOID ARTHRITIS

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Recent studies have redefined the 25-hydroxyvitamin D (25-OHD) reference range (RR) in normal populations; no longer is it seen as winter and summer dependent. PTH data suggest 25-OHD RR is >40 nmol/L. Elderly populations living at higher latitudes are particularly prone to develop 25-OHD insufficiency/deficiency. The design of the study was to examine whether there is an association between biochemical markers of inflammation (such as CRP and ESR), 25-OHD and disease activity in elderly subjects with rheumatoid arthritis (RA).

In the study CRP and ESR were used as biochemical markers of inflammation. IDS's RIA kit was used to measure total 25-OHD concentration (inter-assay CV < 8.5%); alkaline phosphatase (ALP) was also measured as an additional bone marker. Biochemical measurements were performed on fresh specimens during autumn October–November and then again during April–May period.

A complete set of data was obtained on 35 patients with RA; 30 females and 5 males, with the group mean age 63 years.

During Oct–Nov and Apr–May period, 22 (71%) patients had 25-OHD level below RR (mean = 25 nmol/L); in the remaining group mean 25-OHD was 62 nmol/L. In presence of low 25-OHD, mean ESR was 38 compared with 26 in those with high 25-OHD level. Similarly, mean CRP (mg/L) was 24 in presence of low 25-OHD compared with mean CRP of 11 when 25-OHD was normal.

In summary, the data support the idea that inflammatory markers are negatively associated with vitamin D status in RA patients.

TP2.33

HYPERCALCAEMIA AND HYPERPARATHYROIDISM AUDIT IN LITHIUM TREATED PATIENTS

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An association between lithium treatment and hyperparathyroidism is well known. Studies quote an incidence for hyperparathyroidism as high as 15%. In Scotland there are 3 sets of guidelines all recommending serum calcium measurement annually in patients on lithium therapy. An audit was carried out to establish whether raised serum calcium concentration could indicate an increased incidence of hyperparathyroidism in a Glasgow-based population. Over a 6 month period between 2002 and 2003 all samples sent to the laboratory for lithium measurement had an additional bone profile added. If the patient was found to be hypercalcaemic a sample for parathyroid hormone (PTH), vitamin D and repeat bone profile was requested from the general practitioner. Over the sampling period, 853 samples were processed. 27 patients (3.2% of the population studied) were found to have serum calcium >2.6 mmol/l, of these 13 (48%) were found to have transient hypercalcaemia with an isolated

raised calcium value. Two patients had raised PTH values and both of these patients had persistent hypercalcaemia. However, only 11 patients (46%) had a PTH measurement. This audit showed that there is no significant increase in the incidence of hypercalcaemia in this lithium treated population as compared with the general population (incidence 2.5%). The majority of these patients had transient hypercalcaemia probably representing a spurious value secondary to the application of a tight tourniquet. If more evidence becomes available to suggest that long-term lithium treatment may be associated with other bone metabolism disorders it raises the issue whether PTH measurement and/or DEXA scan would be required as part of the routine assessment of patients on long term lithium treatment.

TP2.34

PROSPECTIVE STUDY OF THE EFFECT OF INTRAVENOUS BISPHOSPHONATE ON INR IN PATIENTS TAKING WARFARIN

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Aim: To examine whether patients on Warfarin and receiving Bisphosphonates experienced significant alteration in INR levels. Methods: A prospective study was undertaken in 20 consecutive out-patient and in-patients—between March and May 2004 and who were attending our Metabolic ward for treatment for osteoporosis or Paget's disease and were also taking Warfarin for other indications. Bisphosphonates used were Pamidronate 30 or 45 mg in 500 ml normal saline over 2 h, Zolendronate 2 mg in 100 ml normal saline or Clodronate 1200 mg over 1–2 h having checked that INRs were in the therapeutic range. Basal INRs were noted and any changes at day 1, 3, 5 or 7 for day cases, and at day 1, 2, 3, 4 or 5 for the in-patient group were noted where possible.
Results: No significant adverse bleeding events occurred clinically. There was no significant change either statistically in the INRs before and after Bisphosphonate therapy ($p=0.592$). The average baseline INR was 2.730 (range 1.7–4.3 S.D. 0.706). The mean average post Bisphosphonate INR was 2.835 (range 1.9–3.6 S.D. 0.634) and the mean difference −0.105 (95% CI −0.509–-0.299).
Conclusion: No significant differences in INRs were obtained in the post Bisphosphonate therapy results. Our current practice therefore incorporates less frequent INR monitoring. We proceed with Bisphosphonates if INR is in the therapeutic range and obtains one subsequent test.

TP2.35

ANALYTICAL AND CLINICAL VALIDATION OF AN AUTOMATED HUMAN PARATHYROID HORMONE ASSAY ON THE LIAISON® ANALYZER

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Parathyroid hormone (PTH) measurement is essential to assist in differentiating the causes of hypocalcemia and hypercalcemia. We describe the analytical and clinical validation of the DiaSorin LIAISON® N-tact™ PTH assay, a direct, two site sandwich type assay utilizing chemiluminescene detection.
Analytical sensitivity was <1.0 pg/mL. Functional sensitivity was less than the lowest reportable concentration (2.5 pg/mL). Inter-assay precision, expressed as %CV, was <10%. The mean (± S.D.) percent recovery was 100.5% ± 7.3% (range, 84–108%). Linearity was observed across a range up to 500 ng/L. We found no significant difference between serum and plasma values. No significant differences were found either between fresh samples and samples exposed to five freeze/thaw cycles. Cross-reactivity to PTH fragments was less than 0.1% for all fragments except the 7–84 fragment.
Reference values (median, 2.5th to 97.5th percentile) established in 145 healthy subjects were 32 (7–82) pg/ml. Samples with elevated PTH concentrations ($n=55$) were obtained from patients with primary hyperparathyroidism (HPT), secondary HPT or hypocalcemia of various causes. Samples with low PTH concentrations ($n=42$) were obtained from patients with cancer hypercalcemia or post-surgical hypoparathyroidism. When compared to the DiaSorin IRMA assay, the resulting regression equation was LIAISON=IRMA(1.09)+5.5; $r=0.995$. When plotting PTH against Ca levels, there was a complete separation between HPT, cancer hypercalcemia and hypo-parathyroidism.
In summary, these data demonstrate that the DiaSorin LIAISON® N-tact™ PTH assay is a robust, rapid, accurate and precise tool for the assessment of human PTH that should be very useful in clinical diagnosis and patient management.

TP2.36

VALIDATION OF AN AUTOMATED HUMAN 25 OH VITAMIN D ASSAY ON THE LIAISON® ANALYZER

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The measurement of 25 OH Vitamin D is of growing importance, notably because of the recognition of the increasing incidence of vitamin D deficiency. We have validated the DiaSorin LIAISON® 25 OH Vitamin D assay, based on an antibody and microparticle-based, chemiluminescent immunoassay method.
Assay functional sensitivity was 17.5 nmol/L (=7.0 ng/ml). The time to first result was 40 min. Inter-assay precision, expressed as %CV, was <15%. The mean (± S.D.) percent recovery was 101% ± 13% (range, 82–127%). Dilution linearity was excellent ($r=0.98$). Values remained stable in samples maintained at 4 °C for up to 5 days. There was equivalent cross-reactivity of 25 OH Vit. D2 and D3 and there were no significant differences between the total 25 OH Vit.D concentration by HPLC and the LIAISON® concentration. No significant differences were found either between fresh samples and samples exposed to five freeze/thaw cycles. The equivalence between serum and EDTA plasma samples was demonstrated by least squares regression analysis (for fresh

samples: plasma=serum (0.98)+6.2; $r=0.91$). As for the comparison with the DiaSorin 25 OH Vit. D RIA, the equation was LIAISON®=(0.81)RIA+11.9; $n=329$; $r=0.91$). We measured 25 OH Vit. D in 142 healthy European subjects. All samples were obtained during the late autumn. The median value was 55 nmol/l with a range (2.5th–97.5th percentiles) from 17.5 to 116 nmol/L (7.0 to 46.4 ng/ml).

In summary, these data demonstrate that the DiaSorin LIAISON® 25 OH Vitamin D assay is a robust, rapid, accurate and precise tool for the measurement of 25 OH Vitamin D.

TP2.37

BONE-SPECIFIC ALKALINE PHOSPHATASE IN MEN WITH COELIAC DISEASE OR OSTEOPOROSIS

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Osteoporosis is a known complication of Coeliac Disease (CD). We measured bone-specific alkaline phosphatase (BALP, IDS Ltd, UK) and total alkaline phosphatase (TALP) in serum, and calcium/creatinine ratios (UCa/Cr) in 24 h urine collections from 45 men with CD (some of whom had osteoporosis) and 45 age-matched healthy controls (HC). CD was treated by calcium and gluten avoidance. We also studied 38 age-matched men with untreated Osteoporosis (OP), defined by vertebral fracture.

Between-run CV for the BALP assay was 8.4% at 8.6 μg/L, 2.5% at 12 μg/L and 1.2% at 49 μg/L. Within-run CV was 5.6% at 8.4 μg/L and 2.2% at 33 μg/L. Second samples, taken from 13 HC after 1 year, gave a pooled within-subject CV of 10.1%.

Median BALP was 11.8 μg/L (5.0–33.5) in CD, 8.2 μg/L (5.0–14.0) in HC and 9.5 μg/L (5.7–27.6) in OP. (CD vs. HC, $P<0.0001$; CD vs. OP, $P=0.004$; OP vs. HC, $P=0.05$ (Wilcoxon)). TALP was 80 IU/L (40–153) in CD, 57 (35–91) in HC and 67 (43–274) in OP. (CD vs. HC, $P=0.0002$; CD vs. OP, $P=0.34$; OP vs. HC, $P=0.01$). BALP correlated closely with TALP in CD ($r^2=0.80$) but much less in HC ($r^2=0.24$) (Pearson). The correlation in OP was even weaker than in HC ($r^2=0.11$).

Median UCa/Cr was 0.17 (0.006–0.589) in CD, 0.24 (0.029–0.653) in HC and 0.27 (0.11–0.83) in OP; the difference was significant only between CD and OP ($P=0.05$).

Neither BALP nor TALP fell during treatment of CD.

BALP appears to increase more than TALP in CD, and, unlike TALP, is higher in this condition than in our sample of untreated osteoporosis. Its failure to fall during treatment of CD requires further investigation.

TP2.38

PREVALENCE OF VITAMIN D DEFICIENCY AND SOME OF ITS RISK FACTORS IN REPRODUCTIVE AGE WOMEN IN TABRIZ

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Vitamin D deficiency is recognized as a major health problem contributing to bone disorders particularly osteomalacia and osteoporosis related fracture. The aim of this study was to evaluate the prevalence of vitamin D deficiency in reproductive age women in Tabriz City.

This was a cross-sectional study of 252 women of reproductive age, 15–49 years, from Tabriz City who were randomly selected from the general population. Serum levels of calcium, phosphate, alkaline phosphatase, and vitamin D were measured. Weight and height was measured using seca scale and cotton ruler. Body mass index was calculated based on weight and height. Vitamin D, calcium, phosphate, and alkaline phosphatase were measured by commercially available kits.

The results indicated that the prevalence of vitamin D deficiency in these women was as follows: severe vitamin D deficiency 15.1%, moderate deficiency 15.5%, and mild deficiency 33.7%. BMI defined 3.7% of these women as underweight and 59.8% were suffering different stages of obesity. Only 37.5% had a BMI in the normal range. There was a significant correlation between the serum level of vitamin D and weight ($p<0.05$). There was a decreased level of calcium and phosphate, 8.7% and 3.7%, respectively. Alkaline phosphatase was increased in 1.6%. There was no significant correlation between vitamin D status and calcium, phosphate, or alkaline phosphatase.

In conclusion, vitamin D deficiency is prevalent in a significant percentage of women of Tabriz city. Therefore, intervention with education, food fortification, and sun exposure are recommended to promote improved women's health in Tabriz City.

TP2.39

DEVELOPMENT AND EVALUATION OF AN ASSAY FOR INTACT PARATHYROID HORMONE (PTH) ON THE ABBOTT ARCHITECT® IMMUNOASSAY SYSTEM

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Background and objective: Parathyroid hormone (PTH) is involved in the maintenance of calcium homeostasis, and its measurement plays an important role in the diagnosis and management of calcium metabolism disorders. The purpose of our study was to evaluate an assay in development for the measurement of intact PTH on the Abbott ARCHITECT series of immunoassay analyzers.

Methods and results: The ARCHITECT Intact PTH assay is a two-step immunoassay that utilizes chemiluminescent magnetic microparticle technology. The assay has a reportable range of up to 3000 ng/mL, and may be run in either a routine (30 min to 1st result) or STAT (16 min to 1st result) format. Analytical sensitivity for the assay (95% confidence method) was <0.5 pg/mL. Assay imprecision was evaluated over 15 days using 2 reagent lots using serum panels. Total imprecision at 6.2, 41, 152, and 882 pg/mL was 11.4%, 7.3%, 5.1%, and 4.4%, respectively. Method comparison versus a commercially available immuno-radiometric assay gave the following data (Passing-Bablok): $y=1.05x+2.13$ ($n=180$); r (Pearson)=0.98. No high does hook effect was observed up to PTH concentrations of 400,000

pg/mL, and cross-reactivity versus PTH fragments was <0.01%. Spiking PTH (from 58 up to 2747 pg/ml) revealed mean recoveries of the samples varying from 102 up to 110%.
Conclusions: Based on our data, we conclude that the ARCHITECT Intact PTH assay is sensitive, accurate, and precise. In addition, the availability of a STAT protocol also allows for the rapid, intra-operative determination of intact PTH levels.

TP2.40

ANTI-CCP ANTIBODIES: A NEW SPECIFIC MARKER IN RHEUMATOID ARTHRITIS

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Objective: Anti-cyclic citrullinated peptide antibodies (anti-CCP) are considered to be a highly specific marker of rheumatoid arthritis (RA). The aim of this preliminary study was to assess the serum anti-CCP in patients with RA in relation to other laboratory parameters.
Methods: Thirty patients (women): suspected for early RA-ERA (10), with moderate-MRA (17) to severe-SRA (3) were included in the study. All patients with disease duration 2–28 months were treated with a combination of NSAID and DMARS drugs. Clinical diagnosis was made on the basis of joint pain, swelling and radiological changes. Serum anti-CCP (Euroimmun), RF IgM and IgG (Immundiagnostika GmbH) were determined by ELISA and CRP by high-sensitivity Dade Behring test.
Results: 8 ERA patients were RF IgM positive (57.4 ± 55.9 IU/ml) and 1 was IgG positive (81.2 IU/ml) but positive anti-CCP (>5 RU/ml) were found only in 5 ERA women (170.1 ± 60.1 RU/ml); also in these 5 patients CRP values were higher (4.2 ± 5.1 mg/l). All cases with MRA were positive for RF IgM (22.8 ± 9.6 IU/ml) and only one for IgG (82.1). Similarly among patients with SRA-2 were IgM (55.2 ± 54.5 IU/ml) and 1 IgG positive (46,2 IU/ml). High anti-CCP were found in active form of RA: 11 patients with moderate and 2 with SRA (134.97 ± 83.3 and 167.1 ± 199.4 RU/ml, respectively). However, in patients with remission of the disease anti-CCPAb significantly decreased (0.32 ± 0.39 and 1.90 RU/ml; $p=0.002$). Also CRP values were significantly higher in patients with active RA comparing to those in remission (12.5 ± 1.3 and 42.8 ± 29.3 vs. 2.4 ± 0.97 and 4.5 mg/l; $p=0.0009$).
Conclusions: Assessment of anti-CCP antibodies may lead to better diagnosis, monitoring and prognosis for patients with RA.

TP2.41

DIFFERENTIAL EFFECT OF PPAR AGONISTS ON GENERATION OF HUMAN OSTEOCLASTS IN VITRO

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The peroxisome proliferator-activated receptor (PPAR) regulates the activity of other transcription factors in the adipogenic differentiation and inflammatory response pathways. We investigated the expression of PPAR receptors and the effects of PPAR specific agonists, on osteoclasts formation and function.
Human peripheral blood mononuclear cells (PBMCs) were stimulated with human recombinant RANKL and M-CSF to generate osteoclasts. RNA was extracted at days 0, 7, 14 and 21 and RT-PCR for all three PPAR receptor isoforms demonstrated their expression throughout the culture period. In parallel cultures, PPAR agonists (1×10-9M to 10-5M) were added from the beginning of the culture till day 14, when mature osteoclasts were formed and the number of osteoclasts assessed by counting TRAP positive cells with 3 or more nuclei. PBMCs were also grown on dentine wafers without the addition of any compounds until day 14. Once mature osteoclasts were formed, the PPAR agonists (1×10-9M to 10-5M) were added for 7 days and the extent of resorption was measured.
Activation of all PPAR isoforms with specific agonists (ciglitizone, L165041 and GW9578) resulted in significant dose dependent inhibition of osteoclastogenesis ($p<0.05$). Dose dependent inhibition of osteoclast resorption was observed with ciglitizone, a PPAR-gamma specific agonist ($p<0.05$), whereas L165041, a PPAR-beta specific agonist, resulted in significant dose dependent stimulation of osteoclast resorption ($p<0.05$). GW9578, a PPAR-alpha specific agonist, suppressed osteoclast resorption when 1×10-7M was added to the culture ($p<0.05$).
These data establish a link between PPARs and osteoclastogenesis generated by RANKL and M-CSF stimulated PBMCs, and support a role for differential PPARs signaling pathway in modulation of osteoclasts formation and function.

TP2.42

ANALYTICAL AND CLINICAL EVALUATION OF A NEW ROCHE ELECTROCHEMILUMINESCENCE IMMUNOASSAY FOR THE DETERMINATION OF TOTAL P1NP

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Objective: Analytical and clinical evaluation of the total procollagen 1 amino-terminal propeptide (P1NP) electrochemiluminescence immunoassay on a Roche Elecsys 2010 immunoassay analyzer.
Materials and methods: The analytical evaluation was performed according to standardized protocol based on the concepts of ECCLS documents. Imprecision studies were carried out using control material and human pool sera. Intra-assay imprecision was tested on 20 replicates per analysis for 2 days. Interassay imprecision was tested using control material in duplicate for 10 days and the mean value was used for inaccuracy assessment. Interferences were investigated for hyperbilirubinemia, lipemia and hemolysis. For clinical evaluation, 3 groups of patients: I, secondary hyperparathyroidism due to hemodialysis ($n=30$); II, women on treatment for osteoporosis ($n=26$) and III, patients after bone fracture ($n=11$), were tested along with healthy volunteers ($n=50$).
Results: Imprecision studies yielded intra-assay CVs of 2.4–2.5% and interassay CVs of 2.2–3.4%. Inaccuracy was 6.2–9.2%.

Interferences from triglycerides (<9.8 mmol/L), bilirubin (<750 μmol/L) and hemoglobin (<4 g/L) were undetectable. The medians in groups I, II, III and control group were 338.6, 40.5, 166.1 and 51.7, respectively. With 95%CI, maximal diagnostic efficiency for groups I, II and III were reached at >89.3 ng/ml (specificity 94%; sensitivity 100%), ≤32.5 (specificity 92%; sensitivity 40.7%) and >147 (specificity100%; sensitivity 60%), respectively. The respective AUCs were 0.994, 0.649 and 0.742.
Conclusion: The new Roche electrochemiluminescence immunoassay for P1NP yields precise and accurate results, mostly free from interferences. P1NP could be a useful biochemical tool in assessing bone turnover in patients on hemodialysis and after bone fracture as well as a marker of therapeutic efficacy for osteoporosis.

TP2.43

COMPARISON OF DIASORIN METHODS FOR MEASUREMENT OF 25 OH VITAMIN D WITH THE LC-MS/MS METHOD

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Measurement of 25 OH vitamin D provides the assessment of vitamin D nutritional status. Vitamin D is an essential component in the regulation of calcium and bone metabolism. Vitamin D2 and D3 are used to supplement low levels of vitamin D. Decreased levels may be seen in dietary deficiency, mal-absorption and disease.
The LC-MS/MS method has a stable isotope added to a 0.2-mL plasma sample as internal standard. 25-OH-VitD2 and 25-OH-VitD3 are quantified individually and a sum was used for correlation studies. The RIA 25 OH Vitamin D assay is a direct antibody assay and utilizes the same antibody as the CLIA 25 OH Vitamin D LIAISON® assay. Quantification of 25 OH vitamin D with 90 patient samples by liquid chromatography-tandem mass spectrometry was done by combining the individually measured levels of 25 OH Vitamin D2 and 25 OH Vitamin D3 (Mayo Medical Laboratories, Rochester, MN). This total level was then compared to the 25 OH Vitamin D RIA and the LIAISON® (DiaSorin, Inc, Stillwater, MN).
Comparison of the LC-MS/MS method to the RIA (RIA=0.91(LC-MS/MC)+5.4 ng/mL (r=0.93)) and the LIA (LIA=1.02(LC-MS/MS)+2.0 ng/mL (r=0.90)) yielded good correlations and clinical equivalence in the range from 7–150 ng/mL. These comparisons show that both the RIA and the LIA methods measure 25 OH Vitamin D2 and 25 OH Vitamin D3 and levels compare very closely to the LC-MS/MS method which is considered as a gold standard method for the measurement of 25 OH vitamin D.

TP2.44

HOLIDAYS ABROAD CAN BE PROBLEMATIC FOR THE LABORATORY

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A 29 year old female with MEN 1 and treated with Alphacalcidol and Calcichew following a parathyroidectomy in November 2000 was monitored at regular intervals with PTH and Vitamin D (25-Hydroxycholecalciferol) measurements. Post thyroidectomy her PTH was consistently undetectable and Vitamin D was in the range 74–98 nmol/L (reference interval 25–120 nmol/L) until October 2004 when serum Vitamin D dramatically increased to 1610 nmol/L. She was normocalcaemic and the PTH remained undetectable. Prior to the elevated Vitamin D the laboratory had adopted an automated procedure in preference to the previously long-standing manual extraction method. Assay interference was suspected but there was no evidence for heterophilic antibodies and dilution studies were parallel to the dose response curve. Discussion with clinicians confirmed no change in drug treatment. Serum was sent to the SAS Laboratory at Manchester for confirmation of our results by HPLC analysis. The 25-Hydroxycholecalciferol was confirmed as very elevated but the 1,25 di-Hydroxycholecalciferol was normal at 25 pg/mL (reference range 20–50 pg/mL). This left all concerned mystified until one of the clinicians noted that the patient had recently spent 6 months in South Africa. Increased exposure to sunshine had increased her 25-Hydroxycholecalciferol and the absence of PTH prevented metabolism or toxicity.

TP2.45

CALCIUM, PHOSPHATE AND PARATHORMONE LEVEL CHANGES DURING ORAL GLUCOSE TOLERANCE TESTING

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Eighteen healthy people aged between 21 and 64 years were included in this study which was designed to determine the changes in calcium (Ca), phosphate (P) and parathormone (PTH) levels after oral glucose load. Fasting and 15, 30, 45, 60, 90, 120, 180 min venous blood was collected after a 75 gram oral glucose load. Glucose, Ca, P, alkaline phosphatase, creatinine (Cr), insulin and PTH levels were determined in plasma samples with standard methods. Ca, P and Cr levels were also determined in urine samples at the same time. Wilcoxon test was performed to compare the values obtained at 15, 30, 45, 60, 90, 120, 180th minute with basal values by using SPSS 11.0 statistics program. After oral glucose load, there was no significant change (p>0.05) up to the 45th minute and then a decrease in serum Ca levels was observed. There was an increase in urine Ca/Cr level which was maximal at the 90th minute. A decrease was observed in urine P levels which was in parallel with the decrease in serum P. After a decrease at the 15th minute, an increase in PTH was observed, similar to glucose and insulin peaks. The change in calcium and phosphate levels after glucose load was thought to be related to their second messenger roles in signal pathways of insulin action.

TP3: ENDOCRINOLOGY AND FERTILITY

TP3.01 SELECTED FOR POSTER CLINIC

REGIONAL AUDIT OF ALDOSTERONE AND RENIN INVESTIGATIONS

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Prevalence of primary hyperaldosteronism (PHA) is estimated to be about 10% in the hypertensive population. Laboratories are therefore receiving an increasing number of requests for aldosterone and renin measurements. Practice in the North Thames Region was audited to assess how requests for aldosterone and renin are handled and to propose standards for their appropriate use, especially for screening for PHA.

23 laboratories returned questionnaires; 8 from hospitals with a specialist hypertension clinic. Most have annual workloads of 20–50 requests for aldosterone and renin, with 5 exceeding 200 requests. Most laboratories send their requests to a referral laboratory; few measured aldosterone (3) and renin (4) locally. Most include information about blood pressure, plasma and urine electrolytes and drug therapy, essential for result interpretation, if it has been provided by the requesting clinician.

78% laboratories have protocols for screening for PHA. Submitted protocols indicated wide variation in requirements to withdraw antihypertensive medication prior to screening. Most laboratories use a random aldosterone/renin ratio (ARR) for screening although 22% still use posture studies, which add little information to the random ratio and require an overnight hospital stay if carried out correctly. For confirmation of a raised ARR, 65% of laboratories recommend a repeat ratio after discontinuation of all interfering drugs; 48% carry out posture studies. There were 7 different protocols for posture studies, varying in timing of recumbent and ambulant samples.

Standards proposed include: (1) laboratories should have a protocol for the investigation of PHA; (2) screening for PHA requires measurement of aldosterone, renin and ARR following discontinuation of spironolactone (6 weeks) and β-blockers (2 weeks); (3) a raised ARR should be confirmed after withdrawal of all interfering drugs.

TP3.02 SELECTED FOR POSTER CLINIC

THE EFFECTS OF ROSIGLITAZONE ON INSULIN RESISTANCE AND OXIDATIVE STRESS IN NON-OBESE PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Women with PCOS display a high prevalence of cardiovascular risk factors. Oxidative stress is also accepted as a risk factor for development of atherosclerosis. The aim of this study is to assess the effects of rosiglitazone and metformin on cardiovascular risk factors in non-obese PCOS patients.

Fifty patients with non-obese PCOS and 35 healthy subjects were included this study. Patients were divided into two groups. One group metformin group (MET-group, $n=25$) was treated with metformin, the other group rosiglitazone group (ROSI-group $n=25$) received rosiglitazone for 12 weeks. All measurements were repeated at the end of 12 weeks.

Compared with controls, women with PCOS had elevated free testosterone, androstenedione, DHEA-S and LH. HDL-C and Apo A levels were lower in PCOS patients than control subjects. Lp (a) level was significantly higher in PCOS patients when compared with controls. No significant difference was observed other lipid parameters. Serum MDA, HOMA-IR and AUCI levels were significantly higher while serum TAS level was significantly lower in women with PCOS compared with controls. Serum free testosterone showed a significant positive correlation with MDA and HOMA-IR, and a negative correlation with TAS level in PCOS patients. HOMA-IR, AUCI and MDA levels significantly decreased, TAS level significantly increased after the rosiglitazone treatment. HOMA-IR, AUCI levels significantly reduced, while TAS, MDA levels did not change in MET group. Serum androgen levels and LH/FSH ratio decreased after only ROSI-group.

In non-obese PCOS patients oxidative stress, insulin resistance were increased, rosiglitazone treatment improved these parameters and decreased serum androgen levels.

TP3.03 SELECTED FOR POSTER CLINIC

A COMPARISON OF SERUM CREATININE, CYSTATIN C AND IOHEXOL CLEARANCE FOR THE ASSESSMENT OF GLOMERULAR FILTRATION RATE IN PATIENTS WITH FUNCTIONAL THYROID DISEASE

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Background: Serum creatinine level was found to be higher in hypothyroidism and lower in hyperthyroidism, compared to euthyroid state, whereas cystatin C showed inverse changes. Our study was performed to assess GFR in thyroid disease using iohexol clearance, a 'gold standard' to establish whether either serum creatinine or cystatin C is a true measure of GFR in thyroid disease.

Methods: Ten patients with hypothyroidism and nine patients with hyperthyroidism were recruited. Serum creatinine, cystatin C and iohexol clearance were determined at diagnosis and again when free thyroxine was normalised. Hypothyroid patients were treated with levothyroxine. Hyperthyroid patients were treated with antithyroid drugs or radioiodine.

Results: In patients with hypothyroidism, the mean FT4 ($\pm$S.D.) was 5.4 ± 2.72 pmol/L at diagnosis and increased to 17.48 ± 3.2 pmol/L following treatment. In hypothyroidism, creatinine was 98.1 ± 13.8 μmol/L and on treatment decreased to 87 ± 9.2 μmol/L ($p=0.05$) whereas cystatin C increased from 0.66 ± 0.11 to 0.74 ± 0.15 mg/L ($p<0.05$) and iohexol clearance GFR was 77 ± 9.1 mL/min before and 83.3 ± 14.5 mL/min ($p=0.19$) after treatment. In patients with hyperthyroidism, mean FT4 was 64.4 ± 29.7 pmol/L at diagnosis and decreased to 12.3 ± 2.12 pmol/L and the mean FT3 of 22.7 ± 8.3 pmol/L decreased to

4.7 ± 0.65 pmol/L following treatment. Creatinine increased from 66.7 ± 17.9 μmol/L at diagnosis of hyperthyroidism to 86.5 ± 16.4 μmol/L ($p < 0.001$) following treatment, whereas cystatin C declined from 0.91 ± 0.14 mg/L to 0.68 ± 0.07 mg/L ($p < 0.001$) and iohexol clearance GFR was 105 ± 14.7 ml/min before and 98 ± 16.9 mL/min ($p = 0.15$) after treatment.
Conclusion: There is no significant change in iohexol clearance GFR and creatinine and cystatin C cannot be used to assess GFR in presence of thyroid disease.

TP3.04 SELECTED FOR POSTER CLINIC

IS NEWLY DIAGNOSED HYPOTHYROIDISM ASSOCIATED WITH HYPONATRAEMIA?

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Most clinical guidelines suggest that hypothyroidism is excluded in patients with unexplained hyponatraemia, but little data exist to support the association. This study aimed to establish the relationship which exists in newly diagnosed hypothyroid patients.
All samples sent from primary care to Hull Royal Infirmary between January 2000 and December 2004 and which requested both thyroid function tests and serum sodium were included in the study. The sodium concentrations from all 1001 newly diagnosed hypothyroid patients (776 F, 215 M, median age 64 yrs (IQR 48–75)) during this period were compared with 4875 controls (2975 F, 1900 M, median age 58 yrs (IQR 43–72)) who had normal TSH values in samples collected on the 15th of each month.
The reference intervals (2.5th to 97.5th centile) for the hypothyroid and control groups were 132–144 and 134–144mmol/L, respectively. Multiple regression showed a significant relationship between lower sodium concentration and hypothyroid status (−0.48mmol/L, $p < 0.0001$), patient age (−0.09mmol/L per decade, $p < 0.0001$), and female sex (−0.13mmol/L, $p = 0.046$). Within the hypothyroid group, every 10 mU/L rise in TSH was associated with a 0.14 mmol/L fall in sodium ($p < 0.0001$). None of the hypothyroid group and 2 of the control patients had sodium values <120 mmol/L ($p = 0.76$).
Thus, although hypothyroidism at presentation has a statistically significant effect on sodium concentrations, the magnitude is unlikely to be of clinical importance. Severe hyponatraemia associated hypothyroidism, if it exists, must either be sporadic (and rare) and/or be in patients who are more profoundly hypothyroid than those being routinely diagnosed.

TP3.05 SELECTED FOR POSTER CLINIC

INTERPRETATION OF RESULTS IN THE ASSESSMENT OF ACROMEGALY–A VIEW FROM UK NEQAS

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While primarily focusing on analytical aspects of laboratory testing, EQA surveys also provide a valuable means of auditing the clinical interpretation of laboratory results, highlighting differences in practice that could benefit from improved consensus.
Serum specimens issued to participants in the UK NEQAS for Growth Hormone (GH) in November 2004 formed the basis of one such audit. Participants were invited to interpret their analytical results in the light of three different clinical scenarios: a single random GH determination in a patient with suspected acromegaly and two oral glucose tolerance tests (OGTT) for patients with suspected acromegaly and treated acromegaly. Fifty (50%) of participants returned completed surveys.
Most interpretations offered were appropriate but some were incomplete (e.g. failing to mention IGF-1 as a helpful complementary test in the diagnosis of acromegaly) or positively misleading [e.g. 6% of respondents suggested a random GH level of 8.4 mU/L (~3.2 μg/L) of IS 80/505 in a patient with suspected acromegaly excluded this diagnosis]. Upper limits of the reference interval for random GH quoted by 20 respondents varied from 5 to 50 mU/L (~1.9 to 19.2 ug/L), confirming significant differences with regard to figures quoted and the relevance of reference intervals for GH. Similarly cut-off limits quoted for normal GH suppression during an OGTT varied from 0.2 to 5 mU/L (~0.08 to 1.92 μg/L).
These survey results emphasise the need to improve consensus on use of GH results, which will be facilitated by current international initiatives encouraging universal reporting of GH results in mass units of IS 98/574, the new recombinant International Standard for Growth Hormone.

TP3.06 SELECTED FOR POSTER CLINIC

MEASUREMENT OF GONADOTROPINS AFTER SEX REASSIGNMENT SURGERY IN FIFTY TRANSSEXUAL PATIENTS

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Introduction: Medium- and long-term post-surgical data about the evolution of the gonadal axis in these persons are scarce.
Methods and aims: A total of 395 persons with gender identity disorder have been attended by the first and only public unit in Spain since 1999, and more than 80 genital and non-genital operations have been undertaken. We present the post-surgery hormone therapy used and the evolution of the gonadal axis in the first 50 patients after their sex reassignment surgery; 40 male-to-female transsexuals (MF) and 10 female-to-male transsexuals (FM). Their ages ranged from 18–60 years (mean 26.5 years).
Results:

1. Presurgical levels of gonadotropins were adequately suppressed.
2. Sixty percent of the MF and 8 of the 10 FM developed a pattern of primary hypogonadism (LH>20mU/mL) more than 1 year after surgery, despite maintaining and even increasing doses of post-surgical replacement hormone therapy.
3. The pattern of hypogonadism throughout follow-up (range 6–120 months) improved in 33% of the MF after 18–24 months, but it remained in all the FM.

4. 15% of the MF have stopped attending post-surgical follow-up and in another 20% there is a tendency to self-adjust hormone doses; this was not the case in any of the FM.
5. Reduced bone mineral density was observed in 40% during the pre-surgical phase (mainly in the MF).

Conclusions: Post-surgical hypogonadism is present in persons receiving sex reassignment surgery. It is necessary to individualize therapy, inform the patients of the consequences of stopping treatment and find preventive measures for complications arising during follow-up, especially post-surgical osteopenia.

TP3.07

THE POLYETHYLENE GLYCOL (PEG) PRECIPITATION TEST FOR MACROPROLACTIN IS INFLUENCED BY SERUM IGG AND BIG PROLACTIN

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Precipitation with PEG is a widely employed screening test for hyperprolactinaemia due to macroprolactin. Recovery of prolactin (PRL) in the supernatant >60% after precipitation with PEG 6000 (final concentration 125 g/L) indicates that macroprolactin is not present in significant amounts. When recovery is ≤60% macroprolactin may be present and the lower the recovery the more certain this is. The PEG precipitation procedure is not specific for macroprolactin and confirmation by gel filtration chromatography (GFC) may be helpful. Two cases with discrepant results (PEG precipitation recovery 37%, 39% but no macroprolactin detected on GFC) prompted us to further investigate the specificity of the PEG precipitation procedure. Case 1 had multiple myeloma with an IgG paraprotein of 56 g/L and case 2 HIV infection with serum globulin 62 g/L. Low recovery of PRL after PEG precipitation has been reported previously in subjects with HIV infection and considered evidence that macroprolactin contributes to circulating PRL.
Electrophoresis of serum proteins showed that PEG precipitation largely removed the γ globulin fraction. GFC of redissolved PEG precipitate showed macroprolactin, big PRL and a proportion of serum monomeric PRL present.
Addition of human γ globulin to serum containing only monomeric PRL reduced recovery of PRL after PEG precipitation from mean 86% to 47% (mean globulin 84 g/L).
GFC of 10 further cases of HIV infection with low recovery of PRL after PEG precipitation (25–58%) showed the absence of significant amounts of macroprolactin in 9. Elevated γ globulin (4) or the presence of big PRL (2) probably contributed to the low recovery in some but not all cases.

TP3.08

PRIMARY HYPERPARATHYROIDISM AND DIABETES INSIPIDUS IN A PATIENT ON LONG-TERM LITHIUM THERAPY

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This is the case of an 89 year-old Caucasian female who presented with shingles to the accident and emergency department of Epsom General Hospital, Epsom in 1998.
She is known to have a manic-depressive disorder and had been on lithium therapy for several years at the time of presentation. On admission in addition to her shingles, she complained of nausea, vomiting and constipation.
Initial biochemical investigations showed that she had hypercalcaemia; her adjusted calcium was 3.3 mmol/L (reference range=2.2 – 2.6). Her serum phosphate was within the reference range. She had marginally raised serum urea and creatinine consistent with dehydration. Serum lithium was found to be elevated at a concentration of 1.56 mmol/L (target range 0.6 – 1.0). Her blood intact parathyroid hormone was elevated at a concentration of 28.6 pmol/L (reference range 0.5 – 5.5).
Her 24-hour urine showed hypocalciuria (1.3 mmol/day, reference range 2.57.5). Hypocalciuria is thought to be a feature of primary hyperparathyroidism due to lithium therapy.
Familial hypocalciuric hypercalcaemia is unlikely, as this is a benign condition.
She was diagnosed as having primary hyperparathyroidism due to long-term lithium therapy.
Her lithium therapy could not be stopped. She refused parathyroidectomy for her hyperparathyroidism. She required regular infusions of pamidronate, a bisphosphonate, for her hypercalcaemia.
Five years later in 2003, she was found to have hypernatraemia with raised serum osmolality. A further diagnosis of diabetes insipidus was made. This could be due to the long-standing hypercalcaemia or lithium therapy.
The occurrence of both primary hyperparathyroidism and diabetes insipidus in the same patient possibly due to lithium therapy is an unusual finding.

TP3.09

EVALUATION OF LIPID PEROXIDATION AS AN INDIRECT MEASURE OF OXIDATIVE STRESS IN SEMINAL PLASMA

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It has been proposed that oxidative stress plays an important role in male infertility. The aims of this study were to compare seminal plasma levels of 15-F2t-isoprostane (8-iso-PGF2α), malondialdehyde (MDA), and total (sum of free and bound) homocysteine (tHcy) in normozoospermic vs. asthenozoospermic men, and to examine the relationships between tHcy and lipid peroxidation products.
The study was a case-control study with a simple random sampling. The case group consisted of 15 asthenozoospermic males. This group was compared with 15 normozoospermic men. Seminal plasma levels of 15-F2t-isoprostane and tHcy were measured using commercially available enzyme immunoassay (EIA) kits. MDA levels were determined by the thiobarbituric acid (TBA) assay. The Mann–Whitney *U* test was used to compare two groups. Coefficients of correlation were calculated using Spearman's correlation

analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level.
MDA levels were lower in asthenozoospermic subjects than in control subjects (0.72 ± 0.06 μM vs. 0.40 ± 0.06 μM; $p<0.05$). No differences were seen in 15-F2t-isoprostane levels in asthenozoospermic subjects and controls (65.00 ± 3.20 pg/ml vs. 58.17 ± 4.12 pg/ml; $p>0.05$). Interestingly, tHcy levels were slightly higher in asthenozoospermic subjects than in controls (6.18 ± 1.17 μM vs. 4.8 ± 0.52 μM). Sperm motility was inversely correlated with seminal plasma 15-F2t-isoprostane and MDA levels, respectively ($p<0.05$).
Seminal plasma levels of 15-F2t-isoprostane and tHcy showed no significant differences between normozoospermic and asthenozoospermic men. Sperm motility correlated inversely with seminal plasma levels of 15-F2t-isoprostane and MDA. No relationship was found between tHcy and lipid peroxidation. However, higher sample size is required to confirm these findings.

TP3.10

N-TERMINAL PRO BRAIN NATRIURETIC PEPTIDE (NT-PROBNP) CONCENTRATIONS IN PATIENTS WITH ACROMEGALY

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Objectives: Chronic growth hormone excess may lead to cardiovascular complications and repeated assessments of cardiac function are required in acromegalic patients to diagnose incipient cardiomyopathy. NT-proBNP is a cardiac biomarker having diagnostic and prognostic value for heart failure, acute coronary syndrome and other conditions resulting in myocardial stretch. We therefore assessed NT-proBNP concentrations in patients with active acromegaly.
Materials and methods: 25 active acromegalic patients exhibiting no clinical evidence of heart disease (12 males; 13 females; mean age: 50 ± 14 years) and 49 healthy volunteers were included. IGF-I serum levels were determined by an automated method using an acridinium ester chemiluminescence technology (Nichols Advantage®). Serum NT-proBNP values were determined by electrochemiluminescence assay (Roche® Elecsys® 2010).
Results: Because IGF-I levels are highly age-dependent, their values are expressed as z-scores versus a normal population. The IGF-I z-scores in the acromegalic population were: 4.3 [3.1; 5.4] (median and 2.5–97.5 percentiles). NT-proBNP in healthy volunteers were measured at a value of 35.8 [6.2; 204.2] pg/ml (geometrical mean with 2.5 and 97.5 percentiles). NT-proBNP levels in patients with active acromegaly were not significantly different from healthy volunteers (32.3 [2.2;475.3] pg/ml; $p>0.05$). However, 3 acromegalic patients had NT-proBNP values above the normal range: 746.2, 214.0 and 245.7 pg/ml. After further evaluation review, these 3 patients presented post-ischemic cardiomyopathy and hypertension.
Conclusion: Our results showed no significant difference in mean NTproBNP levels between healthy volunteers and patients with active acromegaly. However 3 acromegalic patients had elevated NTproBNP associated with post-ischemic cardiomyopathy.

TP3.11

PLASMA TOTAL HOMOCYSTEINE CONCENTRATIONS IN INFERTILE WOMEN COMPARED TO REFERENCE VALUES FOR ADULTS

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Homocysteine, a thiol-containing amino acid, is an intermediate in the methionine cycle. An impaired methionine cycle in early pregnancy is a severe disorder; it has been shown that approximately one-third of women with recurrent pregnancy loss have hyperhomocysteinaemia or at least a pathological methionine loading test. Homocysteine is directly toxic to the vascular endothelium: it induces procoagulant activity and reduces thrombomodulin activity.
During the period from 1 January through 31 December 2004, 71 patients were evaluated. Patients were age 30 to 40 years and nonpregnant, with a history of at least one failed IVF procedure and with different gynaecological problems. Plasma total homocysteine was measured by an automated chemiluminescence method (IMMULITE®, DPC, Los Angeles, USA). The assay has a calibration range of 2 to 50 μmol/L, and the manufacturer's recommended reference limits for adults are 5.0 to 12 μmol/L with a median of 7.7 μmol/L.
We found measured homocysteine values to be distributed as follows: <4 μmol/L in 3% of cases, 4–5 μmol/L in 8% of cases, 5–7.5 μmol/L in 38% of cases, 7.5–10 μmol/L in 23% of cases, 10–12 μmol/L in 17% of cases, 13–16 μmol/L in 8% of cases, and >26 μmol/L in 3% of cases.
In our study, we found that plasma total homocysteine levels in infertile women are generally within the reference range. The measurement of homocysteine in infertile women should be further investigated. Abnormal changes in the homocysteine concentration could be an additional risk factor of infertility.

TP3.12

ANDROGENIC STEROIDS IN SALIVA

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The quantitative non-isotopic determination of steroid hormones at very low concentrations is still a real problem for the analytical laboratory. With a newly developed luminescence enhanced enzyme immunoassay the measurement of free steroid hormones in saliva is possible even at very low concentration.
The basic biochemical technology applied is the glow chemiluminescence reaction of alkaline phosphatase conjugate by using an acridin derivative as substrate. The efficient and reproducible chemiluminescence of acridinium ester are combined with an Alkaline Phosphatase catalyzed reaction.
Steroid hormones may be adsorbed to different plastic materials especially in case of low concentrations. Therefore we have

developed a special saliva collection device. These SaliCaps have been tested with Tritium labelled steroid hormones without any non-specific binding to the surface.
We investigated the microplate assays for testosterone and DHEA. We measured the salivary hormones from 78 healthy males. Each volunteer collected 11 saliva samples every 1 h between 7 am to 5 pm. We found the following results:

Analytical sensitivity:	
Testosterone LIA	1.8 pg/mL
DHEA LIA	4 pg/mL
Age dependent ranges:	
Testosterone LIA	
20–30 years	141–206 pg/mL
30–40 years	107–187 pg/mL
40–50 years	101–165 pg/mL
50–70 years	81–148 pg/mL
DHEA LIA	
20–30 years	140–610 pg/mL
30–40 years	162–550 pg/mL
40–50 years	134–350 pg/mL
50–70 years	86–300 pg/mL
Diurnal rhythm: Decrease of morning median values	
Testosterone: 30–50%	
DHEA: 40–60%	

The decrease in concentration with the daytime was higher in younger men compared to older ones.

TP3.13
THE EFFECTS OF HORMONE REPLACEMENT THERAPY ON SERUM LEPTIN, IGF-1 AND IGFBP-3 LEVELS IN POSTMENOPAUSAL WOMEN
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We investigated the influence of hormone replacement therapy (HRT) on the serum leptin, insulin like growth factor-1 (IGF-1) and insulin like growth factor binding protein-3 (IGFBP-3) concentrations in postmenopausal women. In addition, the objective of this study was to determine if serum leptin concentrations were associated with IGF-1 and IGFBP-3.
Thirty-seven healthy postmenopausal women (mean age, 49.1 ± 3.2 years) participated in this study. Women were classified two groups as obese (BMI>25 kg/m2) and non-obese (<25 kg/m2). Serum leptin and IGF-1 and IGFBP-3 levels were assessed at baseline and after 6 months of HRT (conjugated equine estrogen, medroxyprogesterone acetate orally). Leptin and IGF-1 were determined by enzyme-linked immunosorbent assays. An immunoradiometric assay was used to measure IGFBP-3.
Obese group had significantly higher serum leptin and IGF-1 levels (5.08 ± 1.25 ng/ml, 184.8 ± 64.4 ng/ml, respectively), in comparison with the levels of the non-obese women (4.02 ± 0.80 ng/ml, 130.5 ± 48.7 ng/ml, for both of them; $p > 0.01$) before HRT, although there was no significant difference in the IGFBP-3 levels (4372.3 ± 651.5, 4224.8 ± 494.0 ng/ml, $p > 0.05$). Serum leptin (5.96 ± 1.31, 4.82 ± 1.23 ng/ml) and IGFBP-3 levels (4721.1 ± 489.8, 4792.0 ± 807.0 ng/ml) were significantly elevated in both obese and non-obese subjects after HRT. But serum IGF-1 remained unchanged at the end of the study. There was a significant positive correlation between leptin and IGF-1 at baseline while this relation was not correlated after HRT.
Our results show that serum leptin concentrations are significantly higher in obese postmenopausal women than in their non-obese counterparts before and after HRT. HRT is associated with an increase in leptin and IGFBP-3 in postmenopausal women.

TP3.14
AN AUDIT INTO THE REQUESTING OF SERUM BETA HCG FROM THE ACCIDENT AND EMERGENCY DEPARTMENT
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Serum beta HCG has been used to diagnose an ectopic pregnancy, to help diagnose a failing pregnancy and to monitor a woman after a miscarriage with requests often made via Accident and Emergency departments.
An increasing number of requests from this source for serum beta HCG were noted by our Biochemistry department.
A retrospective study was undertaken in conjunction with the department of Obstetrics and Gynaecology into the appropriateness of requesting serum beta HCG via Accident and Emergency with the case notes of 60 patients used in the final data analysis.
For each patient the presenting complaint such as PV bleed, abdominal pain, missed or late period was noted together with the estimation of gestation from the last menstrual period and the outcome of the ultrasound scan on the Early Pregnancy Assessment Unit.
77% of patients presented at 8 weeks gestation or below. 44% presented with PV bleed and 32% with abdominal pain.
In only 7 of the 60 patients (12%) had a request for serum beta HCG been made by obstetric and gynaecological staff in order to assist diagnosis.
53 of the 60 (88%) were not considered necessary in order to make a final diagnosis because all women were given a transvaginal ultrasound usually within two days of presenting to Accident and Emergency and this was the only investigation needed to make a diagnosis in most cases.
As a result of the audit all requests for serum beta HCG will be made by obstetric and gynaecology staff in the Early Pregnancy Assessment Unit which should result in a large reduction in unnecessary requests received by the laboratory.

TP3.15
PREVALENCE OF HYPERPROLACTINAEMIA DUE TO MACROPROLACTIN IN A DGH USING THE DPC IMMULITE 2000 PRL ASSAY
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Macroprolactin is a high molecular weight complex of prolactin (PRL) which has minimal bioactivity in vivo but is a common cause of elevated total serum PRL with some immunoassays.

We determined the prevalence of hyperprolactinaemia due to macroprolactin with the DPC Immulite 2000 PRL assay in our population. All samples with total PRL >700 mU/L using the Immulite assay were investigated for the presence of macroprolactin and determination of monomeric PRL by PEG precipitation and gel filtration chromatography with the Wallac DELFIA assay.

In 12 months 1612 requests for measurement of serum PRL were received and 129 (8%) were considered to be significantly elevated (>700 mU/L). 70 of these cases had not previously been investigated for the presence of macroprolactin. Recovery of PRL after PEG precipitation was >60% in 60 cases indicating that macroprolactin was not present in significant amounts. In 10 cases with total PRL 703–2049 mU/L recovery of PRL after PEG precipitation in the DELFIA assay was <40% and monomeric PRL was <700 mU/L in 9 cases.

We conclude that the prevalence of hyperprolactinaemia due to macroprolactin using the DPC Immulite 2000 PRL assay is 13% of the population with elevated total serum PRL (>700 mU/L). Screening for hyperprolactinaemia due to macroprolactin is essential to avoid unnecessary further investigation, misdiagnosis and inappropriate treatment.

TP3.16

EVALUATION OF POLYETHYLENE GLYCOL PRECIPITATION AS SCREENING TEST FOR MACROPROLACTINEMIA USING ARCHITECT IMMUNOANALYSER

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In this study we performed the PEG precipitation test to determine the incidence of macroprolactinemia in our hyperprolactinemic patients. Therefore, we selected 172 consecutive patients (151 women, 21 men) with PRL>700 mIU/L (mean: 1771 mIU/L; range: 705–25596 mIU/L). PRL was measured by Architect i2000 (Abbott) immunoanalyser both in the serum and in the supernatant obtained by centrifuging the serum treated with PEG 25%.

The incidence of macroprolactinemia was determined using the PRL recovery percentage (R%) as interpretative criterion of PEG test and the 40 and 60% as cut-off: the incidence of macroprolactinemia in hyperprolactinemic subjects was 21.5% ($R\% \leq 40\%$), whereas 74.4% of subjects showed a "true" hyperprolactinemia ($R\% = 60\%$). The correlation test between PRL levels obtained before and after PEG test showed a good association (Pearson coefficient, $r = 0.996$) in the case of the "true" hyperprolactinemic subjects, whereas no correlation was found in the other group.

A recent study suggested considering macroprolactinemic those patients with PRL levels, after PEG test, within the PRL reference interval obtained in normal subjects, whose sera were submitted to the same treatment: in 50 normoprolactinemic subjects we found the PRL values after PEG test ranged between 64 and 453 mIU/L. According to this criterion, the incidence of macroprolactinemia in hyperprolactinemic subjects was 20.3%. In conclusion, the PEG test is absolutely necessary to the laboratory diagnostics of hyperprolactinemia. In 42/172 subjects we were able to evaluate the symptoms ascribed to hyperprolactinemia and the pituitary imaging findings: no significant differences between macroprolactinemic and "true" hyperprolactinemic subjects were found, probably due to the small number of subjects examined.

TP3.17

BIOCHEMICAL DIAGNOSIS OF PHAEOCHROMOCYTOMA: THE ROLE OF PLASMA METADRENALINES

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Phaeochromocytomas are rare neuroendocrine tumours of chromaffin cells that are characterised by autonomous production of catecholamines. Detection and diagnosis are critically dependent on the biochemical confirmation of excessive production by the measurement of plasma or urinary catecholamines and metabolites. Recently, measurements of plasma metadrenalines have been found to have a high diagnostic sensitivity and specificity for detecting catecholamine secreting tumours. We have investigated the role of plasma metadrenalines in the biochemical diagnosis of phaeochromocytoma, particularly in suspected cases with normal or borderline increased urinary catecholamine excretion.

We measured plasma metadrenaline levels along with urinary catecholamines and metadrenalines in 125 patients with strong clinical suspicion of phaeochromocytoma, including 10 subjects with histologically confirmed phaeochromocytoma (7 with sporadic tumours, 3 with MEN2A). Results were compared to 18 healthy normotensive subjects, 15 patients with essential hypertension, 14 with secondary hypertension, 8 with histologically verified non-functioning adrenal masses and 12 patients with a variety of endocrine disorders. Urinary catecholamines and metadrenalines were measured by HPLC with electrochemical detection whilst plasma metadrenalines were determined by immunoassay. No patient with phaeochromocytoma had normal plasma concentrations of both normetadrenaline and metadrenaline. Plasma metadrenalines were increased in all 3 patients with familial tumours in contrast to normal excretion of urinary catecholamines and metadrenalines over multiple samples. Measurement of plasma normetadrenaline and metadrenaline had a sensitivity of 100% with a specificity of 91%.

We conclude that finding normal concentrations of plasma metadrenalines in patients with equivocal urinary catecholamines effectively rules out the presence of phaeochromocytoma and is the test of choice in detecting the disease in high-risk patients with familial endocrinopathy syndrome.

TP3.18

COMPARISON OF ULTRAFILTRATION AND POLYETHYLENE GLYCOL PRECIPITATION FOR DETECTION OF MACROPROLACTINAEMIA

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Macroprolactin is determined by polyethylene glycol (PEG) precipitation. Gel filtration chromatography is the definitive method but is not practical for routine laboratory use. Ultrafiltration (UF) has been suggested as an alternative. PEG precipitation has limitations. PEG precipitates a proportion of monomeric PRL and interferes in some prolactin assays.

We have compared UF with PEG to assess whether it is a viable alternative.

Patient samples with a serum prolactin of >500 mU/L (Bayer Centaur) were investigated ($n=102$). We use 25% PEG 1:1 with sample, spun 30 min at 1500 g. With PEG a recovery cut-off >60% excludes macroprolactin, <40% confirms macroprolactin and 40–60% is equivocal. The same cut-off values have been used for UF. UF was performed with Vivascience 100,000 molecular weight cut-off regenerated cellulose ultrafilters; 500 µl sample was spun at 450 g for 2 h at 15 °C.

Concordant recoveries were: >60% ($n=58$); 40–60% ($n=12$); <40% ($n=4$). There were 22 discordant samples: PEG>60%: UF 40–60% $n=22$ and UF<40% $n=2$; PEG 40–60%: UF>60% $n=2$, and UF<40% $n=1$; PEG<40%: UF 40–60% $n=1$. The line of association was $y=0.7863x+0.0882$. Median UF is 5% lower than PEG [IQR −12 to +1%].

There were two significantly discordant patients PEG vs. UF: 19% vs. 47% and 87% vs. 30%; these patients were investigated by gel filtration. PEG results were confirmed in both cases. One sample had an unusual form of prolactin.

There is correlation between PEG and UF but it is affected by a number of marked outliers. The reasons for this are not known and further investigation is required to validate the UF procedure.

TP3.19

SERUM AND SALIVARY CORTISOL MEASUREMENT IN OBESE WOMEN: CORRELATION WITH PSYCHO-PHYSIOLOGICAL PROFILES

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In obesity the hypothalamic-pituitary-adrenal (HPA) function is frequently impaired and psychopathological alterations are also common. As the assessment of salivary cortisol is useful for studying the HPA axis, 16 overweight/obese women (37.2 ± 1.7 yrs, BMI 31.5 ± 1.4, mean ± SE) underwent serum and salivary cortisol assay at 8, 16, 23 h and at 8 h after 1 mg dexamethasone (dexa) overnight administration. All women underwent psychometric evaluation by S.Q. (Symptom Questionnaire), P.S.E.D. (Pisa Survey for Eating Disorders), P.S.Q. (Pisa Stress Questionnaire) tests.

Serum cortisol and urinary free cortisol (UFC) were measured by a competitive immunoassay (IMMULITE2000 Analyzer, DPC, USA). Salivary cortisol was determined by a solid-phase radio-immunoassay (Coat-A-Count, DPC, USA). Serum cortisol at 08:00 and 23:00 h were 11.8 ± 0.9 and 6.7 ± 1.4 µg/dl, respectively, while salivary cortisol at 08:00, 16:00, 23:00 h were 0.28 ± 0.03, 0.15 ± 0.04, 0.10 ± 0.02 µg/dl, respectively. The UFC excretion was 25.6 ± 3.7 µg/24 h; after 1 mg-dexa serum and salivary cortisol levels were 1.2 ± 0.1 and 0.03 ± 0.005 µg/dl, respectively. Notably, 5 patients had high serum cortisol at 23 h, but after dexa only 1/5 did not suppress serum cortisol. At 23 h 3 patients had high salivary cortisol values, but dexa caused a normal inhibition. A positive correlation between serum and salivary cortisol determinations was found ($r=0.59$, $p<0.02$). In our patients the psychometric evaluation showed a recent onset of anxious and depressive symptoms with a greater attention for their body image. However, there was not a clear psychopathological condition concerning symptoms assessed by tests (anxiety, depression, somatization).

Conclusions: although discrepancies exist between serum and saliva measurements in the HPA study, salivary cortisol, which reflects biologically active steroid, is stress-free, non-invasive and advantageous for studying obese patients.

TP3.20

SIADH SECONDARY TO HEAD INJURY

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A 21 year old female student presented to casualty following a fall while intoxicated, during which she had sustained a fractured wrist and head injury with loss of consciousness. The patient was not taking any medication and denied illicit drug use. A CT scan of the head showed a small haematoma. The serum sodium concentration at this time was 137 mmol/L.

Five days after the initial injury the patient re-presented to casualty with severe vomiting and headache and a serum sodium concentration of 115 mmol/L. The patient was noted to be normotensive and clinically hydrated. Addison's disease was excluded by a short Synacthen test. A repeat CT scan of the head was performed but no abnormality detected.

The serum sodium concentration decreased to 105 mmol/L in the presence of normal renal function, a serum osmolality of 225 mmol/kg, a urine osmolality of 378 mmol/kg and a urinary sodium concentration of 109 mmol/L. Fluid restriction was commenced and the patient responded with the serum sodium concentration increasing to 124 mmol/L after one week and to 127 mmol/L after two months, with resolution of the clinical symptoms. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is characterised by: hyponatraemia, decreased serum osmolality, inappropriately concentrated urine, continued natriuresis (>20 mmol/L), no oedema/hypovolaemia, normal renal and adrenal function and clinical and biochemical response to fluid restriction. A diagnosis of SIADH was supported by a plasma vasopressin

concentration of 0.7 pg/ml. A detectable plasma vasopressin concentration in the presence of hyponatraemia is osmotically inappropriate. The likely cause of the hyponatraemia was SIADH secondary to a head injury, which has been reported to last up to four months.

TP3.21

DAY-TO-DAY VARIATION IN SERUM TOTAL TESTOSTERONE CONCENTRATION IN MEN

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We previously confirmed the marked diurnal variation in total testosterone in men in a single 24-h study and demonstrated that this is maintained into the 7th decade.

To assess day-to-day variability we have measured total serum testosterone concentration in 8 normal men between 0900 h and 1000 h and between 1600 h and 1700 h on the same day on at least 6 days within a 1-month period. We also compared total testosterone concentrations 23.5 h apart in 18 normal men. In one subject, testosterone was measured between 0900 h and 1000 h at random time intervals on 22 occasions over an 8-year period.

Sera were frozen at -20° after separation and measured in batches in a DPC Coat-a Count immunoassay, each subject's samples being assayed in duplicate in the same batch. Inter-batch precision between 6.0 nmol/L and 29.0 nmol/L was <10%.

The mean difference in peak-to-nadir concentrations in AM samples was 30% (12.6%–48%) and in PM samples was 37.4% (15.3%–49%). A difference of up to 33% was observed in samples taken 23.5 h apart. In one subject concentrations ranged from 13.5 nmol/L to 34.5 nmol/L over an 8-year period with no correlation between testosterone and age.

Our results suggest that in normal men serum total testosterone concentration in samples collected under strict conditions may show a marked day-to-day variability both in the morning and the evening. As some of these subjects displayed concentrations below the lower limit of normal on one day while being well into the reference range on another day, it may be prudent to measure total testosterone (along with other relevant hormones) on more than one occasion when results initially might indicate hypogonadism.

TP3.22

PERFORMANCE EVALUATION OF AN IMMUNOASSAY FOR THE MEASUREMENT OF ESTRADIOL ON THE ABBOTT ARCHITECT® ANALYZER

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Measurement of estradiol plays an important role in a wide variety of settings. Because levels can vary widely, estradiol assays should provide accuracy and precision across a wide dynamic range. The objective of our study was to conduct a performance evaluation of the ARCHITECT estradiol assay. The assay is standardized by ID-GCMS and has an auto-dilution range up to 5000 pg/mL. Multi-lot imprecision (total CV) was <7.4% at 45 pg/mL, and <2.6% from 190–600 pg/mL. Functional sensitivity (CV<20%, two instruments, three reagent lots) using human panels was <14 pg/mL. Method comparisons were performed versus two commercially available immunoassays and ID-GCMS. For the ARCHITECT assay vs. ID-GCMS, correlation (r) was 0.99, and average pooled slope was 1.04 ($n=131$). Testing vs.15 samples from an international quality assessment program showed average bias <5%. No significant cross-reactivity or interference was observed for >40 compounds. Recovery of estradiol (in the presence of estrone and estriol) was $100 \pm 10\%$. Reference ranges were established by using prospectively collected samples from 36 menstruating women meeting pre-established clinical criteria to define them as normal. In addition, reference ranges were established in postmenopausal women ($n=72$) and males ($n=101$). Based on our data, we conclude the ARCHITECT Estradiol assay demonstrates excellent accuracy and reproducibility across a clinically relevant range.

TP3.23

INFLUENCE OF DEXAMETHASONE TREATMENT OF RATS ON ADRENAL GLAND ACTIVITY DURING PREGNANCY

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The presence of feto-placental unit and changed hormone milieu during pregnancy influences adrenal glands function.

Stereological and biochemical methods were used to examine the effects of dexamethasone (Dx) on adrenal gland activity of pregnant rats. Blood corticosterone level was measured by 125I RIA Kit. Pregnant rats received subcutaneous injections of Dx, in the doses of 1.0, 0.5 and 0.5 mg Dx/kg b.w./day, during days 16–18 of pregnancy. On days 19 and 21 (24 h and 72 h after the last injection) the dams were sacrificed under ether anesthesia. The controls were injected with saline.

This multiple Dx treatment led to a significantly decreased volume of the cortical zones (ZG by 32%; ZF by 39%; ZR by 27%; $p<0.05$) 24 h after the last dose. The changes resulted from a significant reduction of cortical cells volume and number ($p<0.05$) in all three zones. Three days after the last Dx injection, on day 21 of pregnancy, a significant decline of ZF and ZR volume was recorded, as well as cellular volume in these zones ($p<0.05$). The number of cortical cells was decreased only in ZF. Corticosterone levels in the circulation were significantly decreased ($p<0.05$) in both examined periods as compared to the controls.

While Dx treatment of virgin females led to atrophic changes of adrenal gland cortex 24 h and 72 h after the last application, stereological and biochemical parameters of adrenocortical cells in pregnant rats showed the inhibition of a lower degree comparing to those observed in Dx-treated virgins.

TP3.24

REPRODUCTIVE HORMONAL STUDY IN NORMAL MALE PUBERTY

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The onset of male puberty is clinically characterized by an increase in testicular volume, consequent to reactivation of the hypothalamic-pituitary-gonadal (HPG) axis. An adequate FSH concentration is necessary in the first pubertal stages to induce proliferation and differentiation of Sertoli cells.
The aim of the present study was to evaluate the changes of FSH, LH, T, E2 and PRL during normal male puberty and the relationship between hormonal values and mean testicular volume.
The study included 168 healthy subjects, subdivided by their pubertal development according to Tanner into 5 groups. The subjects all came under our observation for clinical assessment of puberty and were normal stage of pubertal development for their chronological age. Gonad volume was measured using the Prader orchidometer. All subjects and their parents were asked for and provided their informed consent.
Serum FSH, LH, T, E2 and PRL were measured by chemiluminescent microparticle immunoassay (CMIA) (Architect System, Abbott Laboratories, Abbott Park, IL, USA), with detection limits of 0.05 IU/L, 0.07 IU/L, 0.28 nmol/L 66.06 pmol/L, 0.6 ng/mL respectively. ANOVA comparison demonstrated significant differences for FSH, LH, T, E2.
T rises very slowly during G1–G2 and then jumps rapidly for G2–G3 ($P=0.001$) and G3–G4 ($P=0.001$); E2 is similar to T, LH seemingly anticipates the increasing testicular volume, showing a notable increase from G1–G2 ($P=0.001$) and from G2–G3 ($P=0.001$); FSH seems to be the first parameter to notably increase from G1–G3 (G1–G2 $P=0.001$; G2–G3 $P=0.016$), but then drops.

TP3.25

THE EFFECT OF POSTURE AND NEEDLE-PRICK ON PLASMA METADRENALINE LEVELS

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Plasma metadrenalines, normetadrenaline (NM) and metadrenaline (MA), have been advocated as sensitive markers for phaeochromocytoma diagnosis.
The aim of this study was to determine the effects of hypodermic needle insertion and posture on plasma metadrenalines levels to define sample collection conditions.
20 healthy volunteers (8 fasted overnight) had an intravenous cannula inserted while seated. Blood samples were taken immediately (sample1), after 20 min supine (sample2) and 10 min ambulatory (sample3). Plasma was separated and stored at −70 °C without delay. Fractionated metadrenalines were measured by radioimmunoassay (Oxford Biosystems).
There was a significant decrease ($p<0.005$) in metadrenalines on lying and 20 min after needle-prick (sample2). However, all the results were within given "healthy" reference ranges, sample1: NM 9–121, sample2: NM 4–96, (<200 pg/mL), sample1: MA 9–92, sample2: 7–94 (<100 pg/ml). Metadrenaline levels following 10 min ambulation after rest (sample3) were not significantly different from sample1.
The ranges of metadrenaline levels were smaller in the fasting groups for all the three sample collecting conditions (sample1 fasting NM=19–45, non-fasting=6–75; sample2 fasting MA=25–85, non-fasting=5–105).
In conclusion, plasma metadrenaline levels changed significantly in response to needle-prick and shift in posture, but remained within the healthy reference range. A random sample is acceptable for the analysis of plasma metadrenalines as an initial screen. Abnormal results should initiate a repeat sample in fasting state along with rest for 20 min in supine position following cannula insertion.

TP3.26

TOTAL AND FREE HCG AS A BIOCHEMICAL MARKER OF ECTOPIC PREGNANCY

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1% of pregnancies are ectopic. Ectopic pregnancy is associated with a significant incidence of morbidity and mortality. Biochemical monitoring using human chorionic gonadotrophin (HCG) is not satisfactory because serial measurement of hCG over several days is needed to confirm an ectopic and 33% of all ectopic pregnancies will show normal hCG doubling. We investigated the reliability of total hCG , free hCG and progesterone to diagnose an ectopic pregnancy.
355 pregnant patients were investigated. Of these, 170 were viable pregnancies, 25 ectopic pregnancies and 160 spontaneous terminations. Total hCG, free hCG and progesterone were measured on a DPC Immulite 2000. The Kruskal Wallis test was used to determine significance between the groups.
Total hCG, free hCG and progesterone were significantly different ($p<0.0001$) in viable versus ectopic pregnancies.
All markers show statistically significant differences between ectopic and viable pregnancies and spontaneous terminations.
HCG (total and free hCG β) separates ectopic from viable pregnancies but not spontaneous terminations.

TP3.27

DEHYDROEPIANDROSTERONE (DHEA) AND ANDROSTENEDIONE (ANDI): AN ASSESSMENT OF THEIR CELLULAR IMMUNOMODULATORY AND ANTI-RESORPTIVE EFFECTS

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DHEA has been proposed as a corticosteroid- and bone-sparing agent due to its immunomodulatory role and anti-resorptive proper-

ties. The aim of the study was to characterize the cellular mechanisms underlying DHEA's and ANDI's effects. An 'in vitro' study using an osteoblastic cell line was undertaken to investigate the regulation of (1) OPG/RANKL involved in bone resorption (2) a broader spectrum of cytokines/growth factors by Dexamethasone (DEX), and DHEA. OPG and RANKL were measured by ELISA in the supernatant and the mRNA expression determined by real-time PCR. A human cytokine gene array consisting of 268 cytokine-related cDNAs was also used. An intensity ratio between the treated and control arrays was set at >1.5 (up-regulation) or <0.6 (down-regulation). DEX treatment led to an increase in RANKL and a reduction in OPG resulting in a significant 4-fold increase in RANKL/OPG ratio ($p=0.008$), consistent with increased bone resorption. DHEA treatment decreased RANKL expression (76% [3] $p=0.02$) and both DHEA and ANDI at the same concentrations reversed the DEX-induced increase in RANKL/OPG ratio. DHEA led to a down-regulation of several inflammatory cytokines including IL-6 (0.12), IL-4 (0.24), Interferon-gamma (0.44). Confirmatory studies on the cell culture supernatant showed that both DEX and DHEA suppressed the production of the potent pro-inflammatory cytokine; Macrophage Inhibitory Factor: DEX: 45% $p<0.001$, DHEA: 64% $p<0.01$. In contrast to DHEA, DEX down-regulated the expression of several growth factors such as fibroblast growth factor 5 (0.6), vascular endothelial growth factor (0.55), insulin-like growth factor binding protein (0.07). These data support an immunomodulatory role for DHEA without the catabolic effects of DEX.

TP3.28

USE OF LOCI™ TECHNOLOGY TO MEASURE TOTAL Beta-HCG

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We describe the development and initial analytical performance of a homogeneous sandwich immunoassay for measurement of total beta-hCG* (BHCG) using LOCI™ reagents and technology.
The method is based on Luminescent Oxygen Channeling Immunoassay (LOCI™) technology. LOCI™ reagents include two latex bead reagents and a biotinylated anti-BHCG antibody. The first bead reagent (sensibead) is coated with streptavidin and contains photosensitizer dye. The second bead reagent (chemibead) is coated with a second anti-BHCG antibody and contains chemiluminescent dye. A 2 μL sample (serum or plasma) is incubated with chemibeads and biotinylated antibody to form chemibead-BHCG-biotinylated antibody sandwiches. Sensibeads are added and bind to biotin, forming bead-pair immunocomplexes. Illumination of the complexes at 680 nm generates singlet oxygen from sensibeads which diffuses into the chemibeads, triggering a chemiluminescent reaction. The signal is directly related to the BHCG concentration. The method detects intact hCG and free beta subunits, including nicked forms. The analytical range is 0.5–1000 mIU/mL, with no high-dose hook effect observed to at least 3,000,000 mIU/mL. Within-run precision <3%CV with total precision <5%CV were observed over the range 20–500 mIU/mL. No cross-reactivity was observed from LH, FSH, or TSH. Comparison of results from 50 patient samples processed by the new method (Y) and the HCG method on a Dimension® system (X) showed good agreement by linear regression: $Y=0.90(\pm0.01)X-1.6(\pm5.8)$, $r=0.99$, range=1–1000 mIU/mL.
We conclude that use of LOCI™ reagents and technology provides excellent sensitivity, precision, and dynamic range suitable for measurement of beta hCG.
* product under development—not available for sale.

TP3.29

VALUES FOR FIRST AND SECOND TRIMESTER DOWN'S SYNDROME SCREENING MARKERS IN UNAFFECTED PREGNANCIES

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The aim of the study was to establish the reference values for the results of first trimester combined test and second trimester triple test in unaffected pregnancies.
We retrospectively studied 579 women (177 in first and 402 in second trimester of pregnancy), all giving birth to an unaffected baby. The women underwent a nuchal translucency ultrasound measurement and a blood sampling for pregnancy-associated plasma protein A (PAPP-A) and free beta-hCG subunit (fb-hCG) assay in the first trimester and alpha-fetoprotein (AFP), unconjugated oestriol (uE3) and total hCG (hCG) in second trimester of pregnancy. Individual values were expressed as multiple of medians (MoM). Reference limits were established for the entire trimester as 5th and 95th percentile.
For the first trimester markers the values were (mean $\pm$ standard deviation, 5th–95th percentile): PAPP-A: 1.14 ± 0.66, 0.35–2.50 and fb-hCG: 1.37 ± 1.05, 0.41–3.21. The reference limits for PAPP-A were almost the same as cut-off values for Down syndrome, but the 95th percentile for fb-hCG was higher. The values for second trimester were: AFP: 1.09 ± 0.38, 0.58–1,85; uE3: 1.13 ± 0.35, 0.62–1.77 and hCG: 0.97 ± 0.60, 0.23–2.26. Upper reference limits for AFP and uE3 and low reference limit for hCG were lower than cut-off values.
Proper interpretation of the first and second trimester biochemical markers during pregnancy screening requires that laboratories establish and monitor appropriate reference ranges.

TP3.30

A ROLE FOR THE LABORATORY IN THE INVESTIGATION OF SELF-DIAGNOSED HYPOGLYCAEMIA

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A 65 year old woman presented to her GP with self-diagnosed hypoglycaemia. She had documented low blood glucose concentrations using her husband's POCT device. She gave an eight month history of classical symptoms having read about them in literature given to her husband when he was diagnosed with diabetes mellitus two months before her illness began.

After consultation between laboratory staff and her GP, glucose concentrations were measured from blood taken onto filter paper spots during a witnessed, symptomatic episode. They showed hypoglycaemia, as did concurrent meter readings. With these results available she was assessed using a continuous glucose sensor which confirmed hypoglycaemia with a nadir of 2.2 mmol/L.
After an appointment in the Endocrinology Clinic a supervised 24 h fast was arranged. She became symptomatic after 24 h and hypoglycaemia was documented with elevated insulin and C-peptide. A screen for antidiabetic drugs was negative.
Imaging revealed a lesion in the tail of the pancreas consistent with an insulinoma. She underwent a surgical resection of the lesion and made an uneventful recovery with resolution of her symptoms. At histology an insulinoma was confirmed.
Patients often diagnose their symptoms to be due to a low blood sugar. As this case shows, appropriate investigation in primary care, co-ordinated by the laboratory, can indicate whether or not symptoms are due to hypoglycaemia and hence facilitate appropriate referral.

TP3.31

UNDISCLOSED HYDROCORTISONE THERAPY RESULTING IN A MISLEADING URINE STEROID PROFILE REPORT

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At birth, a term baby (consanguineous parents) was noted to have ambiguous genitalia, with clitoromegaly and partially fused labia. Ultrasound showed the presence of a uterus and the adrenal glands were convoluted. Karyotyping confirmed the sex as female. She was otherwise well and was normotensive. Electrolytes and glucose were closely monitored during the first week of life but remained within the normal range. The concentration of 17α-hydroxyprogesterone (aged 24 h) was only marginally raised (49 nmol/L) and was therefore inconsistent with 21-hydroxylase deficiency, the most common cause of virilisation in a female neonate. A Synacthen test showed a blunted cortisol response (30 min: 160 nmol/L) and a highly elevated 11-deoxycortisol (basal: 344 nmol/L), indicating significant 11β-hydroxylase deficiency (CYP11B1).
For confirmation of the diagnosis, a sample was taken for a urine steroid profile. The profile was described as normal and the report stated that CAH was excluded as the cause of the virilisation. Due to the evidence in favour of 11β-hydroxylase deficiency, the urine steroid profile was re-examined and metabolites consistent with this disorder were found e.g. tetrahydro11-deoxycortisol, 6α-hydroxytetrahydro11-deoxycortisol.
Although there were no adverse consequences in this case, it highlights the role of the referring laboratory in determining pertinent drug therapy information before sample referral and indeed in raising awareness among clinicians about pre-analytical factors influencing test results. Some disorders, such as CAH due to 17α-hydroxylase deficiency (see poster TP3.33: 'An atypical presentation of 17α-hydroxylase deficiency'), rely on urine steroid profiling to establish the diagnosis. In these cases such an error may not be detected and may result in an incorrect or delayed diagnosis.

TP3.32

PERFORMANCE EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF FSH ON THE OLYMPUS AU3000i® ANALYZER

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Olympus has developed an assay for the determination of follicle stimulating hormone (FSH) for our new automated immunoassay platform.
FSH is involved in the control of the growth and reproductive activities of the gonadal tissues which secrete male and female sex hormones. Determination of FSH is used to detect hypothalamic-pituitary-gonadal disorders. Gonadal failure is indicated by elevated concentrations of LH and FSH and low concentrations of gonadal steroids.
The Olympus FSH assay is a magnetic particle, chemiluminescent immunoassay for the quantitative determination of FSH levels in human serum using the OLYMPUS ® AU3000i.
We report here results from our development and evaluation of an automated assay for FSH on the Olympus AU3000i® Analyzer.
Lowest detectable level was determined to be 0.02 mIU/ml.
Assay imprecision was characterized over 20 days (80 reps/instrument) according to NCCLS guidelines. Within run coefficient of variation (CV) for Low, Medium, and High human serum pools (6, 30, and 100 mIU/ml) ranged from 1.94 to 2.3%. Between run coefficients of variation ranged from 3.5 to 4.5%.
No significant cross-reactivity was detected from FSH, TSH, hCG, hGH.
The mean recovery for samples tested for linearity was from 94.3% to 96.2%.
No significant interference was detected from bilirubin, haemolysate, Intralipid. Reagents were optimised to avoid RF and HAMA interference.
Based on our evaluation, we conclude that the AU3000i® FSH assay is a sensitive, precise, and accurate method for measuring FSH levels in human serum.

TP3.33

AN ATYPICAL PRESENTATION OF 17α-HYDROXYLASE DEFICIENCY

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A 16 year old boy of tall stature presented with worsening gynaecomastia, of three years duration. On examination, the testes were small for age (~10 mls) and the penis was very short. In infancy he had undergone surgery to correct severe hypospadias. His karyotype was confirmed as 46XY, ruling out Klinefelter's syndrome. Initial investigations demonstrated a low serum testosterone concentration (4.4 nmol/L), elevated gonadotrophins (LH 18.3 IU/L; FSH 10.2 IU/L) and raised serum oestradiol (147 pmol/L).

Furthermore, there was an inadequate cortisol response to Synacthen (30 min: 149 nmol/L) and an exaggerated LH response to GnRH. The gonadal steroids responded poorly to HCG stimulation (testosterone: basal 7.3 nmol/L, post-HCG 8.6 nmol/L; dihydrotestosterone: basal 1.0 nmol/L, post-HCG 1.2 nmol/L) and the concentrations of androstenedione and dehydroepiandrosterone were both low (1.3 nmol/L and <1.0 nmol/L respectively). Renin activity was suppressed to 0.3 ng/ml/hr (reference range 0.5–2.7). Urine steroid profile analysis showed increased excretion of progesterone and corticosterone metabolites, whereas androgens and cortisol metabolites were undetectable. This pattern is consistent with CAH due to 17α-hydroxylase deficiency (CYP17). A homozygous point mutation (T390R) was found in the CYP17 gene.

Gynaecomastia is not usually a feature of 17α-hydroxylase deficiency and furthermore, hypertension and hypokalaemia, the hallmarks of this disorder, were not present in this case. Generally patients with this disorder are identified during the peripubertal period, with primary amenorrhoea and delayed puberty. The majority of patients have been reared as normal females, as the external genitalia are usually female. This atypical, relatively mild case of 17α-hydroxylase deficiency highlights the role of urine steroid profiling in establishing the diagnosis in rare steroid biosynthetic disorders.

TP3.34

EVALUATION OF ESTRADIOL, PROGESTERONE AND LH ASSAYS IN MONITORING OVARIAN STIMULATION COMPARING THE BECKMAN COULTER ACCESS2, BAYER ACS 180SE AND THE BIOMÉRIEUX VIDAS

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In ovarian stimulation the measurements of serum Estradiol (E2), hLH and Progesterone (P) are used accompanied by vaginal ultrasound ovarian scan to determine development of the ovarian follicles, detect endogenous hLH peak and manage follicular to luteal phase evolution respectively. This study was a retrospective analysis of E2, P and hLH during assisted reproduction comparing Access2 and our two current laboratory methods, ACS 180SE and Vidas, and the assessment of the results relative to a successful pregnancy.

For E2 and hLH the Access2 and ACS 180SE methods appear to be equivalent-regression analysis: E2[Access]=1.11E2[ACS]+127, r^2=0.972, n=122 and LH[Access]=0.94[ACS]+0.59, r^2=0.961, n=99. For P two ranges were used for comparison on Access2 and Vidas, regression analysis for values <1.5 ng/ml: P[Access]=1.96P [Vidas]−0.25, r^2=0.408, n=98 and for values >1.5 ng/m/l: P[Access]=0.68P [Vidas]+1.69, r^2=0.796, n=21.

For mono-follicular maturation samples E2 values for Access2 and Vidas methods were similar (250–350 pg/ml). Comparison of hLH in patient follow-up shows similar behaviour for the Access2 and Vidas methods (hLH peak).

P values in follicular phase samples were often higher with Access2 compared to Vidas, and reference values for Access2 could be modified from 1.5 ng/ml to 2 ng/ml for mid-follicular/mid-luteal phase. However, for these very low P concentrations, results should be interpreted in light of potential cross-reactivity and matrix effects, even if this phenomenon does not affect P sample monitoring.

The Beckman Coulter Access2 E2, hLH and P assays can be successfully used to assist the clinician in monitoring ovarian stimulation. As shown in our study reference values for P should be defined to discriminate between follicular and luteal phase.

TP3.35

GLUCOOXIDATIVE STRESS AND SPONTANEOUS ABORTION IN PREGNANT WOMEN WITH DIABETES MELLITUS TYPE 1

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Pregnancies in women with Diabetes mellitus are at risk of increased glucooxidative stress, which could have toxic effects on the developing embryo.

The aim was to evaluate the levels of selenium and glutathione peroxidase (Gl-Px) in pregnant women with Diabetes mellitus type 1 in the first trimester of pregnancy and to establish whether there is a correlation between the degree of glycaemic control and occurrence of spontaneous abortions.

The study included 75 women for 1 year period who were divided in 3 groups:

1st group – 30 with normal outcome;
2nd group – 16 with spontaneous abortion;
3rd group – 29 healthy pregnant controls.

The activity of Gl-Px was determined in erythrocyte hemolysate from EDTA blood. The levels of blood glucose were determined by the Analox GM9 analyzer. The selenium level was determined in whole blood with Li-heparin by atomic absorption.

In all groups the levels of selenium were at the low end of the reference range with no significant difference between them (P>0.05).

There was an increase in the activity of Gl-Px, which is statistically significant in the healthy controls 47.8 ± 13.3 U/g Hb and diabetic pregnancies with normal outcome 48.6 ± 8.4 U/g Hb. There was no statistically significant difference in the activity of Gl-Px in groups 2 and 3 (P>0.05). There was a negative correlation in the levels of pre-prandial glycaemia and levels of selenium in the 2nd group (r=−0.38; P<0.001).

The increased activity of Gl-Px in the women with spontaneous abortion is a result of increased antioxidative defence. The ineffective antioxidant defence, in this group is due to the low levels of selenium and high level of pre-prandial glycaemia.

TP3.36

PERFORMANCE EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF LH ON THE OLYMPUS AU3000i® ANALYZER

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Olympus has developed an assay for the determination of luteinising hormone (LH) for our new automated immunoassay platform.
Determination of LH is useful for the diagnosis and confirmation of hypothalamic–pituitary–gonadal disorders and can be used for the treatment of infertility in women and prediction of ovulation.
The Olympus LH assay is a magnetic particle, chemiluminescent immunoassay for the quantitative determination of LH levels in human serum using the OLYMPUS ® AU3000i.
We report here results from our development and evaluation of an automated assay for LH on the Olympus AU3000i® Analyzer.
Lowest detectable level was determined to be 0.04 mIU/ml.
Assay imprecision was characterized over 20 days (80 reps/instrument) according to NCCLS guidelines. Within run coefficient of variation (CV) for Low, Medium, and High human serum pools (6, 30 and 90 mIU/ml) ranged from 1.7 to 1.93%. Between run coefficient of variation ranged from 4.6 to 5.4%.
No significant cross-reactivity was detected from FSH, TSH, hCG, hGH.
The mean recovery for samples tested for linearity was from 93% to 108%.
No significant interference was detected from bilirubin, haemolysate, Intralipid. Reagents were optimised to avoid RF and HAMA interference.
Based on our evaluation, we conclude that the AU3000i® LH assay is a sensitive, precise and accurate method for measuring LH levels in human serum.

TP3.37

LEPTIN VALUES IN HEALTHY MACEDONIAN POPULATION

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In order to obtain reference values for the leptin hormone in the Macedonian population, an immunoenzyme method was established. The investigation was performed using plasma from a reference group chosen according to the IFCC criteria. Height, weight, blood pressure and body mass index (BMI) were measured, as well as cholesterol, trigylcerides, urea and glucose in all subjects. The results show that leptin reference values depend on the gender. Mean values and standard deviations for leptin were 4.42 ± 4.12 ng/ml, with an upper limit of 12.66 ng/ml in males aged 18–58 years and 7.64 ± 7.90 ng/ml, with an upper limit of 23.44 ng/ml in females aged 19–63 years. The leptin reference values could be presented also in relation to the BMI: in the female up to 14.5 ng/ml with BMI from 18.5 to 24.9; up to 26.2 ng/ml with BMI from 25 to 29.9; up to 35.0 ng/ml with BMI from 30 to 34.9 and up to 39 ng/ml with BMI above 35. In the male population: up to 8.57 ng/ml with BMI from 18.5 to 24.9; up to 8.56 ng/ml with BMI from 25 to 29.9 and up to 9.83 ng/ml with BMI from 30 to 39.
Serum leptin in healthy controls displays a highly positive correlation with body mass index for both sexes and therefore, could be considered as a very important indicator for the quantity of body fat. The other examined parameters in this group of subjects were within the reference limits.

TP3.38

RE-INVESTIGATION OF PATIENTS WITH ANDROGEN INSENSITIVITY SYNDROME USING URINARY STEROID PROFILING

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Androgen Insensitivity Syndrome (AIS) is a disorder resulting from numerous mutations in the androgen receptor gene in poorly masculinised males. AIS must be distinguished from a defect of testosterone synthesis such as 17 ketosteroid reductase deficiency and 5α reductase (5AR) deficiency in which testosterone may be elevated but conversion to dihydrotestosterone is reduced.
Prior to the availability of full biochemical tests and gene sequencing, patients with AIS had insufficient investigations to differentiate the causes. Many patients had gonadectomy due to the risk of malignancy, making reassessment difficult. A urinary steroid profile was performed in adult patients with AIS (CAIS, $n=32$, PAIS, $n=7$) to investigate those that may actually have 5AR deficiency. Urine was also collected from 2 patients with presumed 5AR deficiency and 12 normal controls.
Ratios of Tetrahydrocortisol (THF) to allo THF and 5 β Aetiocholanolone (aetio) to 5 α Androsterone (andro) were used to assess 5AR activity. In 10/39 patients the ratio of THF/aTHF was greater than 4.4 (normal range 1.4–4.4), and in 50% of these patients the ratio was greater than 17. 7 of the 10 patients with an increased THF/aTHF also had an elevated ratio of aetio/andro (normal range 0.6–1.6). Generally the THF/aTHF ratio showed greater elevation than the aetio/andro ratio within the same patient. 7 patients were also found to have an elevated aetio/andro ratio without an elevated THF/aTHF ratio.
4 new cases of 5AR were found in these patients. This study showed that a urine steroid profile was able to detect patients with 5AR deficiency when blood hormone measurements were not possible.

TP3.39

INTRAINDVIDUAL VARIATION IN SERUM HORMONE CONCENTRATIONS IN HEALTHY INDIVIDUALS

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This study was designed to assess the biological variability of serum hormones in healthy individuals. Non-fasting blood samples were taken from 10 healthy individuals at approximately weekly intervals for 6 weeks. These serum samples were analyzed for free triiodothyronine (fT3) and free thyroxine (fT4) by a competitive immunoassay, and thyroid stimulating hormone (TSH) by sandwich immunoassay using an ADVIA Centaur (Bayer) system. Intact parathyroid hormone (iPTH) and insulin-like growth factor-1 (IGF-

1) were measured by sandwich immunoassay using the Nichols Advantage system.
The intra-individual coefficients of variation (CVI) for serum free T3, free T4 and IGF-1 were lowest at 4.7%, 4.8% and 9.4%, respectively. The CVI for TSH and iPTH were highest at 25.1% and 25.9%, respectively. This data along with the inter-individual variation were used to calculate the index of individuality (II) (ratio of intra-individual to inter-individual variation). All hormones except, iPTH and TSH had a marked individuality (II<0.6).
This study shows that for some hormones like free T3 and IGF-1, which have narrow intra-individual variations and marked individuality, population based reference ranges are insensitive to detect early abnormalities.

TP3.40

INVESTIGATION INTO POSSIBLE CAUSES OF INTERFERENCE IN SERUM TESTOSTERONE MEASUREMENT IN WOMEN

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Aims: To see whether there is a relation between urine steroid metabolite concentrations and the degree of interference in serum testosterone measurement in women.
Background: Testosterone immunoassays may give spuriously high results in women. The presumed interference is removed if testosterone is extracted into an organic phase before being measured, and is thought to have no clinical significance "*per se*". Our laboratory uses an extraction method to check all serum testosterone concentrations in samples from women which are greater than 3.0 nmol/L on initial direct assay (Abbott Architect).
Subjects: Women who had a blood sample referred to Hope Hospital Clinical Biochemistry laboratory for measurement of serum testosterone concentration. The study received ethical approval.
Methods: Testosterone results over a 6-month period were used to recruit subjects. A difference (direct minus extracted testosterone) of less than 1.0 nmol/L was used to define a control group. A difference of 2.5 nmol/L or more was used to define a group with interference. A random urine sample for steroid profile analysis by GLC was obtained from patients in both groups.
Results: 10 patients were recruited into the interference group, and 10 into the control group. Comparison of steroid/creatinine ratios for 22 individual steroids revealed no obvious difference between the two groups. The interference group, however, had a higher ratio of androgen to cortisol metabolites.
Conclusions: The interference is associated with higher ratio of androgen to glucocorticoid metabolites, which in fact may have clinicophysiological import. This study has so far failed to identify a single metabolite responsible for the interference.

TP3.41

COMPARISON OF OESTRADIOL (E2) ASSAYS ON THE ROCHE E170 AND PERKIN-ELMER AUTODELFIA IMMUNOASSAY SYSTEMS

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Direct steroid immunoassays are an analytical challenge, particularly at low concentrations. E2 exemplifies this, most physiological values being sub-nanomolar. As the importance of the non-classical actions of E2 in both sexes becomes more evident, measurement of E2 at low levels is required. In men and post-menopausal women, values less than 200 pmol/l are typical, whilst in children and women down-regulated for IVF, values less than 100 pmol/l are common. We compared E2 values in sera of 40 normal male volunteers using the Roche E 170 and Perkin-Elmer AutoDelfia immunoassay systems. We determined precision profiles from duplicate samples using these and other sera. Functional sensitivities for both assays were obtained using Ekins plots. Individual recovery experiments at approximate values of 100 pmol/l were performed by mixing 0.1 ml pooled serum, calibrated in each assay, with 0.4 ml of 40 male sera studied in both systems. Functional sensitivity of the Roche E170 under the conditions employed was 2.5 pmol/l, and for the AutoDelfia, 17.5 pmol/l. E2 concentrations in the male sera studied ranged from 77–225 pmol/l, considerably above the sensitivity of both systems. Recovery of E2 in the Roche assay was lower (80+7% S.D.) than for the AutoDelfia (92+8% S.D. $p<0.01$). Linearity was not compared as the assay diluent was found to have measurable E2. Within batch precision was <10% for both instruments at 50 pmol/l, with reasonable agreement in the range studied ($r=0.93$). There was a mean negative bias of 7 pmol/l for the E170, independent of concentration. Both assays seem sensitive and precise enough for this application but require validation by an independent reference method.

TP3.42

LEPTIN LEVELS IN HUMAN BREAST MILK AND ITS RELATIONSHIPS BETWEEN MATERNAL HORMONAL STATUS

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Leptin, the obese (ob) gene product, is an adipocyte-derived hormone that is involved in the regulation of energy homeostasis and is present in human breast milk. The aims of this study were to determine whether breast milk leptin levels change during lactation and to assess the relationships between milk leptin levels and maternal hormonal status. Breast milk samples and venous blood samples were obtained from healthy lactating mothers at 1–3 days (colostrum) or at 12–180 days (mature milk) after birth. Milk samples were analyzed for leptin. Serum samples were analyzed for leptin, insulin, prolactin, cortisol and estradiol. The

leptin level in colostrum was 2.3 ± 0.3 pg/ml ($n=37$), significantly ($p<0.001$) higher than the leptin level, 0.9 ± 0.1 pg/ml ($n=95$), in mature breast milk. Mature milk leptin levels were stable during the 6 month lactation period, at 12–180 days after birth. Regression analysis revealed a significant positive correlation between mature milk leptin levels and serum leptin ($r=0.762$; $p<0.001$) and serum insulin ($r=0.311$; $p<0.01$), but not serum prolactin, cortisol and estradiol levels in breast feeding mothers during the period of 12–180 days after birth. On the other hand, there was a significant positive correlation between colostrum leptin levels and serum leptin ($r=0.425$; $p<0.05$) and cortisol ($r=0.455$; $p<0.01$) levels, but not serum insulin, prolactin and estradiol levels in breast feeding mothers during the period of 1–3 days after birth. In conclusion, these data demonstrated that (a) leptin level in colostrum is higher than in mature milk, (b) leptin levels in mature milk remain stable during the lactation period, at 12–180 days, and (c) leptin levels in colostrum and mature milk are influenced differently from maternal hormonal status.

TP3.43

RAISED 5HIAA CAUSED BY NON-PRESCRIBED 5-HYDROXYTRYPTOPHAN SUPPLEMENTS

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We present the case of a 30-year-old man who complained of mid-abdominal ulcer-like dyspeptic symptoms of several years duration. He experienced facial flushing after drinking alcohol or coffee and had occasional diarrhoea. He was taking no prescribed medication. Blood investigations were unremarkable other than a low red-cell folate of 211 ng/mL (reference 280–800) and a borderline raised urea of 8.2 mmol/L (reference 2.5–7.5). An upper GI endoscopy was normal as was a biopsy sample for a Helicobacter Pylori (urease test). The urine revealed a 5HIAA value of 392 μmol/24 h (reference<52). The patient was a cigarette smoker and carcinoid syndrome was suspected.

Imaging tests were performed to locate the tumour. CT of the thorax, abdomen and pelvis were unremarkable as was an US scan of the liver. The patient was offered an Indium Octreotide scan but this was declined.

It subsequently became evident that the patient had been self-medicating with 5-hydroxy-L-tryptophan (5HTP) supplements for irritable bowel syndrome during the week prior to his urine collection. He was advised to stop the supplements and refrain from serotonin-containing foods for 2 weeks and then repeat the urine collection. His urinary 5HIAA returned to normal at 30 μmol/24 h.

5HTP is decarboxylated to 5-hydroxytryptamine (serotonin) and converted to 5HIAA. 5HTP is produced commercially by extraction from the seeds of the African plant Griffonia Simplicifolia and has been used clinically to treat depression for many years. Patients must be advised to stop supplements prior to 5-HIAA testing. This initial abnormal result led to unnecessary patient anxiety and distress.

TP3.44

EVALUATION OF A NEW CHEMILUMINESCENT IMMUNOASSAY FOR INSULIN ON A FULLY AUTOMATED ANALYZER

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Objective: The objective of this study is to evaluate the performance of a new assay, ARCHITECT® Insulin (under development at Abbott Laboratories), for quantifying insulin in a routine laboratory situation.

Methods: This kit utilizes the chemiluminescent immunoassay. It is a one-step assay (sandwich) on the ARCHITECT instrument system, which is fully automated analyzer and can be linked to an automated sample-handling system. We carried out performance evaluation including precision, accuracy, sensitivity, linearity and correlation.

Results: The calibrators of this assay were made to reference to WHO standard. Within-run and Between-run CVs were 1.1–1.3%, 1.1–1.7% (3 level concentrations), respectively. Analytical sensitivity was 0.5 μU/mL. Recoveries were 99.5–108.2% for serum, 94.4–100.5% for plasma (EDTA). The dilution linearity was preserved up to 273.5 uU/mL. There was no interference from free bilirubin (up to 19.4 mg/dL), conjugated bilirubin (up to 20.9 mg/dL), hemoglobin (up to 765.0 mg/dL), turbidity (up to 2800 FTU). No high dose hook was observed at least up to 30,000 uU/mL. The regression equation between the present method (ST AIA-PACK IRI, EIA) (x) and the ARCHITECT (y) was: $y=0.832x+0.068$ ($r=0.99$, $n=100$: serum of patients). The throughput was 200 tests/h. The time required to obtain the sample result was 29 min.

Conclusion: ARCHITECT® Insulin is easy to use and fully automated. It is an accurate, fast and high throughput kit. In terms of good performance with rapidity and easy operation, this kit would be useful at clinical laboratories and hospitals for the measurement of insulin.

TP3.45

PERFORMANCE EVALUATION AND LOT-TO-LOT CONSISTENCY OF THE ABBOTT ARCHITECT® PROGESTERONE ASSAY

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Measurement of progesterone is useful in a wide variety of clinical settings. These include determining the cause of infertility, tracking ovulation, and monitoring the health of a pregnancy. Because progesterone levels are sometimes monitored over time, consistency of assay results across multiple reagent lots is especially relevant. The purpose of our study was to evaluate the performance

characteristics and reagent lot-to-lot consistency for the Abbott ARCHITECT Progesterone assay. Assay imprecision was evaluated over a 20 day period for three controls (low, medium, high) and a human serum dilution panel using three reagent lots and two instruments (two runs per day, two replicates per run). Target progesterone levels and total imprecision were: low=0.9 ng/mL, ≤5.6%; medium=5.0 ng/mL, ≤2.7%; high=21.7 ng/mL, ≤2.3%; dilution panel=251.4 ng/mL, ≤2.2%. Using these data, reagent lot-to-lot variation was also calculated. Percent difference across the reportable range (expressed as percent difference of mean values) was: low=6%, medium=2%, high=1%, dilution panel=5%. Assay sensitivity (95% confidence method) for three reagent lots was <0.1 ng/mL. Method comparison studies versus the Immulite progesterone assay were performed with three ARCHITECT Progesterone reagent lots using human serum samples (n=158). Slope and intercept (Passing-Bablok) values ranged from 0.88 to 0.89, and −0.031 to −0.032, respectively; r=0.99. Based on these data we conclude the ARCHITECT Progesterone assay is sensitive and precise, and provides consistent results throughout the reportable range and across multiple reagent lots.

TP3.46

MACRO FSH: ELEVATED SERUM FOLLICULAR STIMULATING HORMONE (FSH) DUE TO AN FSH-IGG COMPLEX

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High molecular mass (macro) forms of circulating thyroid stimulating hormone, luteinising hormone and prolactin have been described resulting from the formation of hormone-IgG complexes. We investigated a patient with elevated serum Follicle Stimulating Hormone (FSH) for the presence of a macro FSH. Serum FSH levels were undetectable during two pregnancies, but returned to high levels after delivery. Maximum serum FSH was 78.9 IU/L using the Perkin-Elmer Wallac DELFIA. In further experiments with the DELFIA assay using a sample with FSH 32.6 IU/L, 28% of FSH immunoreactivity remained after adsorption of serum with protein A-sepharose (controls 102–110%, n=9) and 34% remained after precipitation with polyethylene glycol (controls 88–94%, n=10), indicating the presence of an IgG-FSH complex. 92% of FSH immunoreactivity remained after treatment with heterophilic antibody blocking tubes. On gel filtration chromatography of a further sample with FSH 22.2 mIU/L the serum FSH immunoreactivity eluted with a molecular mass of 207 kDa confirming the presence of a high molecular mass form of FSH. After 4 M urea treatment used to dissociate FSH complexes, the majority of the immunoreactivity eluted in the same position as FSH in control serum with molecular mass 69 kDa. Results of FSH assay in 6 widely used commercial immunoassays were variable; Bayer ADVIA Centaur 3.2 IU/L, Abbott Architect 5.3, DPC Immulite 8.6, Roche Elecsys 8.8, DELFIA 22.2. These data indicate that the elevated FSH in this patient was due to an FSH-IgG complex (macro FSH). The possibility of macro FSH should be considered whenever elevated FSH is not consistent with the clinical evaluation.

TP3.47

REFERENCE RANGE FOR ANDROSTENEDIONE ON IMMULITE (DPC) COMPARED WITH RIA (DSL)

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Measurement of androstenedione is used in the assessment of women with hyperandrogenism. Recently, an automated chemiluminescent method on the Immulite has become available, which may replace the older radioimmunoassay method (RIA).

The aim was compare the RIA method with the DPC Immulite method and to establish local reference ranges for the newer method.

Serum (227 males and 204 females) less than 3 days following collection and kept at 4 °C was randomly selected from anonymized outpatient samples and stored at −20 °C until assayed. Inclusion criteria: age 18–65, ambulatory (outpatient blood collection center). Exclusion criteria: requisition for androgens.

Method comparison was assessed by linear regression. Parametric and non-parametric reference ranges were established using Excel software.

The slope and intercept of the correlation graph were 1.05 and 3.2 nmol/L respectively (r=0.927). Our reference range upper limits, for both men and women, were surprisingly much higher than those suggested by the DPC kit insert (female: 1.0–11.5 nmol/L; male: 2.1–10.8 nmol/L). We found a clear stratification for women into two age groups: 18–44.9 yr (5–33 nmol/L) and 45–65 yr (1.6–15.8 nmol/L) (non-parametric method). The 95 central percentile of males (18–65 years old) will have serum androstenedione within 3.5–15.4 nmol/L (parametric method).

The Immulite method gives higher androstenedione values than the DSL RIA method. Women might have higher "normal" androstenedione levels than expected, and these levels decrease significantly after 45 years of age. In men, androstendione levels decrease more steadily with age.

TP3.48

RADIOIMMUNOASSAY (RIA) METHOD FOR SALIVARY TESTOSTERONE: REFERENCE RANGES AND WITHIN-SUBJECT BIOLOGICAL VARIATION

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Testosterone in blood is largely bound to SHBGs and albumin. It is the unbound fraction of testosterone which is considered to be the biologically active and correlates more closely with the physiologically effective level of steroid hormone. It is postulated that the free fraction or unbound fraction interacts with specific receptors in the target cells to exert their hormonal effects.

The salivary testosterone assay has been developed from a currently used in-house method at the Glasgow Royal Infirmary. The sensitivity of this assay is 25 pmol/L and the intraassay and interassay analytical CVA are 9% and 15%, respectively. The concentrations for adult men (n=99) ranged from 216–1370 pmol/L

(mean 404 mol/L) and for adult women ($n=73$) ranged from <25–149 pmol/L (mean 88 pmol/L). For children, in girls aged 10–16 years ($n=19$) concentration ranged from <25–134 pmol/L and in boys aged <11 years ($n=35$), concentration ranged from <25–44 pmol/L and for boys 11–16 years old ($n=63$), concentration ranged from <25–1340 pmol/L.

We also studied the intra-individual or within-subject biological variation (CVI) in 6 healthy adult females and 5 healthy adult male volunteers over 21 times (time of collection 0900–1200 h) in a 6 month period. The CVI in the male volunteers was 34% and in the female volunteers was 42%. These results indicated that there is a high degree of within-subject variation in the salivary testosterone concentrations in healthy volunteers. Saliva sampling offers a non-invasive stress-free and painless method for the long term or repeated sequential sampling schedules and enable collection of samples at specific times in the day. Data however will have to be interpreted carefully in the context of the high CVI for individual patients.

TP3.49

RELATIONSHIP BETWEEN METHOD MEANS AND ID-GCMS REFERENCE METHOD VALUES IN THE UK NEQAS FOR OESTRADIOL

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A reference measurement system for steroid hormones has existed for three decades, yet its impact on the trueness and comparability of clinical assay systems has been slight. The traceability requirements of the IVD Directive have brought this issue into sharp focus. This study audits changes in method characteristics between 2002 and 2004.

Three unprocessed human serum pools containing only endogenous hormone were assayed for oestradiol using isotope dilution gas chromatography mass spectrometry (ID-GCMS) at the University of Gent steroid reference laboratory. They were first distributed in the UK NEQAS service during 2002 and then again at the end of 2004. On both occasions, most methods showed a degree of positive bias at the lowest concentration (137 pmol/L) and negative bias at higher concentrations of oestradiol. In 2002, regression slopes (method mean vs. ID-GCMS target value) for individual methods ranged from 0.653 to 1.106 and intercepts from −21 to +124 pmol/L with an all-laboratory result of ALTM=0.78GCMS+22. In 2004 regression slopes ranged from 0.66 to 1.025 and intercepts from −41 to +71 with an all-laboratory result of ALTM=0.85×GCMS+24. This improvement was largely due to the Bayer Centaur method group improving from MLTM=0.88×GCMS+38 38 to MLTM=1.025×GCMS+0.3, and the growth in use of the Roche E170 modular system (MLTM=0.92×GCMS+22). The other major method group (DPC Immulite 2000) retained a low slope of 0.7. The spread of results within and between method groups was not markedly different.

These data are encouraging, in that two manufacturers have methods in widespread use with acceptable trueness. Others need to embrace the reference measurement system and be open and transparent about their IVDD traceability.

TP3.50

ESTABLISHMENT OF NORMAL VALUES FOR SEX HORMONES DURING DIFFERENT PHASES OF THE MENSTRUAL CYCLE ON THE ABBOTT ARCHITECT® ANALYZER

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During a normal menstrual cycle, serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and progesterone can vary widely between cycles for the same woman, as well as between different women. Reliable reference values based on the local population are important for correct interpretation of laboratory results. The purpose of our study was to determine reference values for these hormones throughout the menstrual cycle using the Abbott ARCHITECT system. For 20 volunteers (ages 20–36), with normal cycles and no use of oral contraceptives, samples were taken every day of their cycle. Volunteers received three vaginal ultrasounds (days 10, 13, and 1 or 2 days after ovulation) to measure follicular and corpus luteum development. Hormone levels were measured using the respective ARCHITECT assay, and synchronized to the LH peak. Median and 95th percentile values (not shown in this abstract) were determined as follows: early follicular, FSH (6.4 IU/mL), LH (6.1 IU/mL), progesterone (0.2 ng/mL), estradiol (41 pg/mL); late follicular, FSH (4.7 IU/mL), LH (8.5 IU/mL), progesterone (0.2 ng/mL), estradiol (122.8 pg/mL). LH peak, FSH (12.8 IU/mL), LH (63.6 IU/mL), progesterone (1.0 ng/mL), estradiol (182.8 pg/mL); early luteal, FSH (5.6 IU/mL), LH (12.2 IU/mL), progesterone (5.4 ng/mL), estradiol (85.4 pg/mL); mid-luteal, FSH (3.1 IU/mL), LH (6.2 IU/mL), progesterone (11.9 ng/mL), estradiol (135.0 pg/mL); late luteal, FSH (2.8 IU/mL), LH (3.8 IU/mL), progesterone (4.0 ng/mL), estradiol (87.2 pg/mL). Median prolactin values for this population were 9.8 IU/mL. Based on our study, we have been able to establish detailed reference ranges that should aid in the interpretation of results for these reproductive hormones.

TP3.51

CORRELATION BETWEEN SPERM QUALITY PARAMETERS AND SEMINAL PLASMA ANTIOXIDANTS STATUS

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In the etiology of male infertility, there is growing evidence that damage inflicted to spermatozoa by reactive oxygen species (ROS) plays a key role. The aim of present study was to assess the antioxidant status of seminal plasma by measuring total antioxidant capacity (TAC) and activities of catalase and superoxide dismutase (SOD) in men with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic males.

This work was a case-control study with a simple random sampling. The case group was consisted of 46 men with seminal parameters

abnormalities that divided into 3 categories: asthenozoospermic ($n=15$), asthenoteratozoospermic ($n=16$) and oligoasthenoteratozoospermic ($n=15$). The control group consisted of 25 males with normozoospermia. Catalase activity was measured by spectrophotometeric method. Commercially available colorimetric assays were used for measuring SOD activity and TAC.

TAC evaluation showed significantly lower values in the total case group ($n=46$) versus control group (1.05 ± 0.04 mmol/ml vs. 1.51 ± 0.07 mmol/ml, $p<0.05$). Catalase activity also showed significantly lower values in the total case group ($n=46$) versus control group ($14.40 \pm$ 0.93 U/ml vs. 21.33 ± 1.50 U/ml). But this difference was not significant for SOD activity (5.31 ± 0.56 U/ml vs. 6.19 ± 0.83 U/ml). Both catalase activity and TAC in asthenozoospermic, asthenoteratozoospermic, oligoasthenoteratozoospermic subjects were significantly lower than normozoospermic males, but SOD activity did not show a significant difference between these groups. Both catalase activity and TAC showed a positively significant correlation with progressively motile sperms and normal sperm morphology. These correlations with SOD activity were not significant.

In summary, we concluded that decreasing in seminal plasma antioxidant status, especially catalase activity and TAC, may have significant role in etiology of sperm abnormality.

TP3.52

RELATIONSHIP BETWEEN METHOD MEANS AND ID-GCMS REFERENCE METHOD VALUES IN THE UK NEQAS FOR TESTOSTERONE (FEMALE RANGE)

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A reference measurement system for steroid hormones has existed for three decades, yet its impact on the trueness and comparability of clinical assay systems has been slight. The traceability requirements of the IVD Directive have brought this issue into sharp focus. This study audits changes in method characteristics between 2002 and 2004.

Three unprocessed human serum pools containing only endogenous hormone were assayed for testosterone using isotope dilution gas chromatography mass spectrometry (ID-GCMS) at the University of Gent steroid reference laboratory. They were first distributed in the UK NEQAS service during 2002 and then again at the end of 2004.

On both occasions, most methods showed a very marked degree of positive bias, higher at the lowest concentration of testosterone. In 2002, regression slopes (method mean vs. ID-GCMS target value) for individual methods ranged from 0.937 to 1.420 and intercepts from −0.12 to +1.27 nmol/L with an all-laboratory result of ALTM=1.084GCMS+0.575. In 2004 regression slopes ranged from 0.845 to 1.802 and intercepts from −0.039 to +1.345 with an all-laboratory result of ALTM=1.061×GCMS+0.651. Most methods showed similar characteristics in the two distributions, but the Bayer Centaur method group changed from MLTM=0.937×GCMS+0.871 to MLTM=0.8455×GCMS+1.011. Immulite methods had increased slopes despite the recent re-formulation. The Roche E170 modular system not evident in 2002 gave MLTM=1.1975×GCMS+0.6. Once again the DPC Coat-a-Count and Orion Spectria RIA methods seemed to give the least biased results. The spread of results within and between method groups was not markedly changed.

These data are not encouraging; manufacturers need to embrace the reference measurement system and be open and transparent about their IVDD traceability.

TP3.53

GMEC EVALUATION OF SEVEN OESTRADIOL ASSAYS

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We have, with the help of off-site laboratories, evaluated seven oestradiol assays (Abbott, Bayer, Beckman, DPC, Perkin Elmer, Roche and Tosoh) used in UK laboratories. Particular attention was paid to the ability of the assays to measure low concentrations of oestradiol.

Imprecision was assessed using four serum pools and Bio-Rad Liquichek control material. The pool with an all method mean value of 152.5 pmol/L gave a range of results between 107.4 and 174.8 pmol/L with between-assay CV of 3.7–19.5% compared to the pool with a mean of 1166 pmol/L which gave a range of 978–1343 pmol/L with between assay CV of 3.1–44.1%. Measurements made with Liquicheck material were not consistent with those made with serum pools which demonstrates the importance of using patient samples when assessing assay performance.

Method bias was assessed using UK NEQAS samples. All methods showed some bias against the UK NEQAS all method mean ranging from +10.5% to −33.4% for an ALTM<250 pmol/L and +14.6% to −21.7% for an ALTM>250 pmol/L. Those methods calibrated against isotopic dilution gas chromatography mass spectrometry (Abbott, Bayer and Roche) showed a positive bias when results were above 250 pmol/L.

Data from in-house serum pools suggests that some methods have significant cross-reaction with synthetic oestrogen.

Parallelism for all methods was acceptable in the range studied, however positive intercepts were seen with the Perkin Elmer $y=0.94x+47.26$ and Beckman $y=0.96x+48.8$ assays and negative intercept with the DPC $y=1.01x-21.97$ assay.

TP3.54

COMPARISON OF SIX AUTOMATED ASSAYS FOR TOTAL AND FREE PROSTATE SPECIFIC ANTIGEN (PSA) AND THEIR REACTIVITY TOWARDS THE WHO (96/760) REFERENCE PREPARATION

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Objective: To improve assay comparison an international reference preparation which approaches the molecular heterogeneity of PSA in the circulation (90% complexed to ACT and 10% free PSA) has been devised. Many manufacturers of automated PSA assays have referenced their assays to this WHO standard.
The purpose of the present study was to assess the responsiveness of the WHO standard in various assays for free and total PSA and to compare their performance on clinical specimens with different PSA concentrations.
Methods: 70 serum samples and the WHO PSA 96/760 standard were measured. Total and free PSA were measured on the Access (Beckman), Architect and AxSym (Abbott), E170 (Roche) and Immulite (DPC). Total and complexed PSA was measured on the Centaur (Bayer).
Results: All assays measured T-PSA and 3 out of 5 measured F-PSA close to the expected WHO standard value. Slope varied from 1.0–1.2 for T-PSA and 1.1–1.8 for F-PSA.
Agreements of values from the tested assays for T-PSA and F-PSA in patient samples were excellent. Differences in slopes between all assays were less than 10% for T-PSA and less than 20% for F-PSA and r^2 varied from 0.94 to 0.99.
Conclusions: The tested assays correlated well with each other in patient aliquots and all measured total PSA reasonably close to the assigned WHO standard value. There were larger differences in free PSA values measured in the WHO standard.

TP3.55

INTRA-OPERATIVE INSULIN MEASUREMENT DURING RESECTION OF INSULINOMA

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The majority of patients with adult-onset organic hypoglycaemia have a single benign insulinoma. Currently the protocol for treating patients with insulinoma involves pre-operative imaging and angiography with, if necessary, Ca2+-stimulation of the pancreas followed by surgical excision which may be guided by intra-operative ultrasound of the tumour. However multiple foci of hypersecretion are found in 10–20% of patients and are more common in those patients with malignant insulinoma or MEN1. We have investigated the use of intra-operative insulin measurements in this setting.
Two cases of insulinoma presented with a single defined lesion on pre-operative imaging. Insulin, C-peptide, proinsulin and split-proinsulin (Ins and C-pep, Perkin-Elmer DELFIA, Pro and Split-Pro, local assay) were analysed on samples taken after induction but before enucleation of the tumour, and at 5, 10, 20 and 30 min after removal of the hypersecreting tissue.
Pre-operative analyte levels in each patient were; insulin 1370 pmol/L and 430 pmol/L; C-peptide 2550 pmol/L and 2960 pmol/l; Pro-insulin 47 pmol/L and 87 pmol/L; Split-proinsulin 50 pmol/L and 41 pmol/L. Insulin levels decreased by at least 90% in both patients by 20 min with the other analytes showing lower falls of 40–60% consistent with their longer half-lives. Histology confirmed resection of insulinoma in both cases and post-operative glucose levels normalised. The importance of careful control of plasma glucose concentrations was demonstrated in one of the cases after a bolus of glucose given following resection led to a rebound in plasma insulin to 230 pmol/L.
These results display the ability of intra-operative insulin measurements to monitor the clearance of hypersecreting tissue whilst demonstrating its requirement for careful control of blood glucose concentrations during the operation.

TP3.56

INTRA-OPERATIVE PTH MEASUREMENT DURING TOTAL PARATHYROIDECTOMY FOR TERTIARY HYPERPARATHYROIDISM

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Intra-operative PTH measurement has been extensively investigated as an adjunct to the surgical treatment of uniglandular primary hyperparathyroidism. We have examined its use in prediction of successful clearance of hyperplastic tissue in patients with tertiary hyperparathyroidism.
A consecutive series of seventeen patients had bilateral neck dissection and total clearance of parathyroid tissue without autotransplantation. Median pre-operative PTH levels were 910 ng/L(range 170–2420 ng/L, Nichols Advantage). PTH was measured prior to the removal of the first gland then at 5 min after the removal of each gland and at 10 min after removal of the last gland using the Nichols QuiCk-Pak assay system. PTH levels one month post-surgery were compared to intra-operative levels.
After surgery PTH levels fell into 3 groups; 12/17 patients PTH fell below the lower limit of normal (LLN<9 ng/L); 3/17 patients PTH fell below 300 ng/L (NK/DOQI Stage V renal disease target); and in two patients remained elevated at 341 ng/L and 1324 ng/L. In patients with post-op PTH<LLN the median intra-operative decline in PTH was 75% (range 60–86%) at 5 min and 81% (range 70–90%) at 10 min after removal of the last gland. In those five cases with post-op PTH>LLN the median decline was 45% (range 27–71%) at 5 min and 59% (range 50–77%) at 10 min. The operative failures had the highest absolute PTH levels at 10 min (370 ng/L and 290 ng/L). There was no difference in time taken in surgery between the two groups or the profile of PTH fall during the operation.
Patients who are not cured at surgery appear to be identified by a fall in PTH≤65% in addition to a high absolute level of PTH at 10 min.

TP3.57

CHRONIC OPIUM AND CIGARETTE SMOKING MODULATE PITUITARY HORMONES AND PERIPHERAL BLOOD

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Aim and design: Opium is smoked for pleasure or as a medication by some people in Iran. It is a complex mixture of 40 different alkaloids such as morphine and many impurities. Although it is well established that opioids or tobacco affect many physiological functions of human, to our knowledge there is no study looking to these effects in opium and cigarette smokers. To assess that, we investigated the circulating level of Prolactin, TSH, LH, FSH,

testosterone, and peripheral blood cell counts of male opium smokers who also smoke cigarettes ($n=23$, aged 28.4 ± 4.1 years) and compared with corresponding values for nicotine abusers ($n=12$, 15–25 cigarettes/day) or healthy control group ($n=20$) of same age.

Findings and conclusions: Our results showed 87% of the opium dependents and 42% of the nicotine dependents groups had high prolactin ($P<0.002$). In addition, there was a positive correlation between the dose of opium and plasma prolactin level of opium dependents ($R=0.748$, $P<0.001$). Low FSH was detected in 43.5% of the opium smokers and 50% of the cigarette smokers with normal LH and testosterone levels. TSH of the opium smokers was also lower than the other two groups ($P<0.002$). Platelet ($P<0.05$), and erythrocyte counts and corresponding hemoglobin and hematocrit of opium smokers were significantly ($P<0.001$) lower than the other two groups, without changes in the other erythrocyte indexes. In conclusion, present data indicate chronic opium and cigarette smoking may synergistically influence pituitary hormones production through an effect on different neuropeptide produced locally or systemically. In addition, opium may lead to a state of anemia among opium smokers.

TP3.58

MODIFICATION OF ARTERIAL MECHANISMS AND ENDOTHELIAL FUNCTION IN PCOS

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Introduction: Polycystic ovary syndrome (PCOS) is associated with several metabolic abnormalities that are predictors of cardiovascular disease and type II non-insulin dependent diabetes mellitus (NIDDM) and therefore atherogenesis.

Aim: The purpose of this study was to determine whether or not arterial mechanics and endothelial function are modified in PCOS. A rat model of PCOS was used to assess the mechanical properties of the aorta in vitro and endothelial and vascular smooth muscle in vitro.

Results: There was a significant reduction in aortic compliance and increased stiffness in PCOS rats compared to controls. Relaxation induced by Acetylcholine (ACh) was significantly diminished in PCOS rings compared to control rings (68% vs. 84%; $p=0.05$), endothelium independent relaxations with sodium nitroprusside (SNP) were significantly increased in PCOS (PCOS 104% vs. control 87%; $p=0.05$) and in the presence of L-nitroarginine methylester (L-NAME), ACh-mediated relaxation was reduced in control rings relative to PCOS rings (11% vs. 32.5%; $p=0.001$). PCOS rats, treated with mifepristone, had increased levels of luteinising hormone (LH) compared to control rats ($p=0.04$) and had higher levels of testosterone and insulin, although these results were not significant. Rats treated with mifepristone also showed arrest of follicular growth and increased rate of atresia.

Conclusion: we have demonstrated that in the PCOS rat model, endothelium dependent relaxation is impaired, endothelium independent relaxation with SNP is increased and the L-NAME insensitive component is increased compared to controls. The precise mechanism for this action is as yet unknown and warrants further study.

TP3.59

AUDIT OF THE OUTCOME OF THYROID FUNCTION TESTS IN HOSPITALISED PATIENTS

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Nonthyroidal illness (NTI) is frequently accompanied by alterations in circulating thyroid hormone concentrations. The prevalence of one or more abnormality of thyroid function tests (TFT) in patients with acute medical illnesses has been reported from 40 to 70%. It has been suggested that requesting TFT is only worthwhile in hospitalised patients with at least three symptoms or signs related to a thyroid disorder. We performed an audit to assess the outcome of TFT in relation to the number of symptoms and signs of thyroid disorders in hospitalised patients.

The hospital notes of 192 patients who had TFT requested were reviewed (81 males and 111 females; age range: 17 to 98 years). 171 patients (89%) had <3 symptoms/signs and 21 (11%) had 3 or more symptoms/signs. Of the 171 patients with <3 symptoms, 21 (12%) had abnormal TFT but only one patient (0.6%) had a thyroid disorder (primary hypothyroidism). Of the 21 patients with 3 or more symptoms/signs, 4 (19%) had abnormal TFT and 2 patients (9.5%) had thyroid disorders (1 primary hypothyroidism and 1 hyperthyroidism). One patient with non-specific TFT abnormalities was wrongly diagnosed as having hyperthyroidism.

Our audit shows that requesting TFT in hospitalised patients with low clinical suspicion for thyroid disorders has a low yield (1.2%) and is wasteful of resources. In addition, indiscriminate TFT requesting may lead to the wrong diagnosis of a thyroid disorder being made. TFT should only be requested in hospitalised patients who have at least 3 or more symptoms and signs or if the admission diagnosis is directly related to a possible thyroid disorder.

TP3.60

PERFORMANCE EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR TOTAL THYROXINE (T4) ON THE OLYMPUS AU3000i® ANALYZER

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Determination of Total T4 can be used for the diagnosis and confirmation of thyroid disorders: detection of hyperthyroidism, detection of primary and secondary hypothyroidism and monitoring of TSH-suppression therapy. The Olympus Total Thyroxine assay is a magnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroxine levels in human serum using the OLYMPUS AU3000i®. We report here results from our development and evaluation of this automated assay for Total Thyroxine on the Olympus AU3000i® analyzer. The assay is calibrated with nine

gravimetrically prepared calibrators (0–240 μg/L). The lowest detectable level was determined to be 1.4 μg/L. Assay imprecision was characterized over 20 days (80 reps/instrument) according to NCCLS guidelines. Within run coefficient of variation (CV) for the Low, Medium, and High level human serum pools (20, 81, and 156 μg/L) ranged from 0.9 to 1.4%. Total inter-assay imprecision ranged from 1.5 to 2.3%. Specificity was determined by testing 9 analogues of T4 spiked into the zero calibrator. Cross-reactivity was <1% for 6 of the tested compounds. Levels of 30% were observed with D-T4 and Tetrac while 6% cross reactivity was observed with Triac. The mean recovery of samples tested for linearity was 95.5%. No significant interference was detected from bilirubin, haemolysate, γ-Globulin, Intralipid, and HSA. Based on our evaluation, we conclude that the Olympus AU3000i® Total Thyroxine assay is a sensitive, precise, and specific method for measuring Thyroxine levels in human serum.

TP3.61

HYPERTHYROIDISM AND CARDIOVASCULAR RISK FACTORS

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Background: Hyperthyroidism is a common disorder affecting multiple systems in the body and cardiovascular effects are most striking. Beside the lipids and lipoproteins, recently total homocysteine (tHcy) and C-reactive protein (CRP) are considered as additional risk factors for CAD.
Objectives: (1) To investigate tHcy and CRP measured by high-sensitivity methodology (hsCRP) in patients with hyperthyroidism on thiamazol treatment, (2) To assay lipids and lipoproteins in the same investigated groups.
Methods: The study patients were 15 women with hyperthyroidism and 17 euthyroid, age-matched female controls. A serum aliquot was taken after an overnight fast. tHcy (reference range 5–15 μmol/L) was measured with IMx fully automated fluorescence polarization immunoassay (Abbott), CRP (reference range ≤3 mg/L) with latex-based immunoassay (Bio-kit, Spain). Triglycerides, cholesterol and HDL were measured on Beckman Synchron CX 4 analyzer using the commercial tests of RANDOX. LDL was calculated according to Friedwald. Parametric data were tested by Kruskal-Wallis one way ANOVA. P values <0.05 were considered statistically significant.
Results: Significant differences between groups were found only for triglycerides ($p<0.05$). No significant differences were observed for cholesterol ($p=0.497$), HDL ($p=0.590$) and LDL ($p=0.761$). Values for hsCRP were increased (1.84 mg/L vs. 1.43 mg/L) but not significantly ($p=0.824$). A similar pattern was seen for tHcy (11.18 μmol/L vs. 10.44 μmol/L), $p=0.824$.
Conclusion: No increment of cardiovascular risk factors other than triglycerides was observed.

TP3.62

THE EFFECTIVENESS OF AN INTERPRETATIVE COMMENT ON THYROID FUNCTION TEST REPORTS

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Aims: To ascertain if the use of a new interpretative comment on thyroid function test reports suggesting thyroid antibody measurement had elicited testing of thyroid antibodies in these patients. Secondly, to determine whether the workload for thyroid antibody measurement had increased following the use of the new interpretative comment.
Background: Several studies into sub-clinical hypothyroidism have found that the risk of developing overt hypothyroidism is increased in the presence of anti-thyroid antibodies. In June 2003 we implemented a new comment for patients with raised TSH and normal FT4. This comment was: "If patient not on T4, results suggest sub-clinical hypothyroidism, warranting measurement of thyroid antibodies, if not already done".
Subjects: Data were collected for all patients with the comment on their test reports and all patients that had thyroid antibodies measured for a year before and a year after the introduction of the comment.
Results: 40% of patients with the new comment on their thyroid function test report subsequently had their thyroid antibodies measured. The majority of these requests were carried out within two months of the initial report. The workload for anti-thyroid antibodies increased by 87% in the year following implementation of this comment. Anti-thyroid antibodies measurement as a result of the addition of this interpretative comment were more likely to be positive (64%) than those requested randomly (26%).
Conclusions: The addition of the interpretative comment had a positive effect.

TP3.63

HOOK EFFECT IN THYROGLOBULIN RADIO AND CHEMILUMINESCENT IMMUNOASSAY

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The diagnosis of metastatic neck nodes may require Thyroglobulin (Tg) measurement in the washout from fine needle aspiration biopsy (FNAB). Often the aspirate presents high concentrations, and therefore provokes the hook effect. Levels inducing such an effect may be furthermore observed in serum after node laser-ablation.
At the Hospital of Reggio Emilia, FNAB and ablation techniques are in use, and samples containing hook levels are usual. In order to identify such cases, each sample is assayed undiluted and diluted 1/2. In the case of hook, the diluted value will be higher than the undiluted. The hook cases are then diluted in the range 1/10–1/10,000, allowing the determination of the definite concentration (and also the construction of a dose-response curve up to mg/ml, including hook paradox).
With the aim to determine the hook thresholds in different multi-site immunoassays, the chemiluminescent system Access2 (ICLA) and the radiometric DiaSorin (IRMA) have been tested. They behave as follows: low-level comparison: ICLA=1.48 IRMA – 1.5; high-level: ICLA=0.25 IRMA+4652. Imprecision: at 1, 10, 100 ng/ml, %CV are

3.0, 2.0, 1.5 for ICLA, and 5.5, 3.7, 2.7 for IRMA. Sensitivity (ng/ml): Analytical: 0.05 ICLA and 0.15 IRMA; Functional: 0.1 ICLA and 0.3 IRMA.
12 FNAB aspirates and 3 ablation sera were diluted and assayed with the two systems, showing that the hook threshold for ICLA is around 4000 ng/ml, while for IRMA around 1500 ng/ml.
It is concluded that a diluted sample must be assayed in parallel with the undiluted when FNAB or ablation samples are submitted to Thyroglobulin immunoassay.

TP3.64

THE VALUE OF TPO AND TSHR ANTIBODIES IN GRAVE'S DISEASE

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Requesting for thyroid antibodies has increased dramatically over the past few years. Generally the first line test is thyroid peroxidase antibodies. Negative results are sometimes assumed to rule out all forms of autoimmune thyroid disease including Grave's although there are reports that 20–50% of these patients have negative TPO antibodies.
We have reviewed the thyroid peroxidase antibody results in patients with positive TSH receptor antibodies and a diagnosis of Grave's disease over a period of 18 months.
TPO antibodies were measured using the ELISA method from Binding Site. TSH receptor antibodies were measured using the ELISA kit from RSR LTD.
Between January 2003 and the end of June 2004, 112 thyrotoxic patients (confirmed by suppressed TSH and raised free T3 but not on thyroid supplements) had TSH receptor antibodies greater than 1 U/L (reference range 0–1 U/L). Of these 65 (58%) had raised TPO antibodies and 47 (42%) had negative TPO antibodies (reference range <40 U/mL).
Our conclusion is that TSH receptor antibodies should always be measured where thyrotoxicosis is proven and Grave's disease is suspected regardless of the results of TPO antibody assessment.

TP3.65

GUGGULSTERONE AND ABNORMAL THYROID FUNCTION TESTS: CASE REPORT

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A previously fit and well 31 year old male presented with a 6 week history of dry mouth and sweating. He had lost 2 kg in weight over the last 3 months. There were no other symptoms typical of either hypo- or hyper-thyroidism. His only currently prescribed medication was zopiclone, but he had been taking the herbal supplement Guggulsterone 150 mg/day for 3 months. Investigations revealed a low TSH 0.059 mu/L; (0.1–4), low Free Thyroxine 7.0 pmol/L (10–22), low Total Thyroxine 35 nmol/L (60–160) and normal Total T3 1.4 nmol/L (1.1–2.8). The patient was advised to stop taking the Guggulsterone. Six weeks later his symptoms had improved and his thyroid function tests had returned to normal (TSH 1.25 mu/L, Free Thyroxine 12.1 pmol/L, Total Thyroxine 68 nmol/L and Total T3 1.9 nmol/L).
The gum resin of the Guggul tree (Commiphora mukul) has been used in Ayurvedic Medicine for over 2000 years to treat a variety of ailments including obesity, disorders of lipid metabolism and inflammatory conditions. The ethyl acetate extract of this gum resin, Guggulipid, received regulatory approval in India in 1987 for use as a lipid-lowering agent. The active compounds in this extract are believed to be the isomers E- and Z-guggulsterone. In the UK Guggulsterone is widely available in a variety of herbal supplements.
There are numerous studies of Guggulsterone's lipid lowering effects in humans; however research into the effects of guggulsterone on the thyroid has predominately been confined to animal studies. These suggest guggulsterone has a thyroid stimulatory action and promotes conversion of thyroxine to T3.
This case serves as a reminder to consider natural or herbal remedies when faced with abnormal results.

TP3.66

FALSELY INCREASED ELECSYS FT3: EIGHT CASES IN EIGHT MONTHS

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Free triiodothyronine (FT3) measurements may be used to confirm hyperthyroidism and for monitoring thyroid hormone therapy. Between May and December 2004, we suspected an interference in eight sera with increased FT3 (from 7.1 to 12.1 pmol/L, upper reference limit 7.0 pmol/L) and normal or increased TSH, both measured with an Elecsys assay.
We measured FT3 in these samples with a two-step RIA not affected by anti-T3 autoantibodies. We used the Ria-gnost FT3 assay (Schering CIS bio international). We ran this assay in a two-step format using, in the first incubation step, the incubation buffer from the two-step Gammacoat FT4 assay (DiaSorin). In all sera FT3 values between 3.1 and 6.0 pmol/L were within the reference interval (3.1 to 6.45 pmol/L). These findings confirmed the interference in the Elecsys FT3 assay.
Anti-T3 autoantibodies and heterophilic antibodies are two possible causes of falsely increased Elecsys FT3 results. We checked the sera for anti-T3 antibodies using a radio-binding assay. All samples were negative except for one serum which was weakly positive. We checked the sera for heterophilic antibodies using the HBT tubes from Scantibodies. Treatment of the sera in HBT tubes did not decrease significantly Elecsys FT3 results, and thus interference from heterophilic antibodies is unlikely.
Interference from drugs, such as nonsteroidal anti-inflammatory drugs, has been described in the past as a cause of falsely increased FT3 results. Medical treatments of the patients and other causes of interference are currently being studied in an attempt to identify the cause of this rather frequent interference (0.2% of FT3 measurements performed during this period).

TP3.67

ASSESSMENT OF THE NEW xMAP TECHNOLOGY IN THE DETECTION OF THYROID ANTIBODIES

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AIM OF THE STUDY. Multiplex bead array immunoassays are increasingly being used not only for research but for routine purposes as well. The era of random access multiplex bead instruments performing a variety of tasks is not far away. In this context we tried to evaluate the analytical characteristics of a new commercial multiplex immunoassay for the detection of thyroid antibodies and compare it with a traditional ELISA method.
METHODS AND PATIENTS. The instrument being used was the Luminex 100 which can differentiate signals for 100 parameters in a single sample with the use of xMAP technology: beads that emit in a different way due to a mixture of two fluorescent dyes. The Quanta Plex Thyroid Profile (INOVA Diagnostics) is a homogeneous multiplex immunoassay using beads covalently linked with thyroid peroxidase (TPO) and thyroglobulin (Tg). We tested 30 sera from patients diagnosed with autoimmune Hashimoto's thyroiditis either with active disease or in remission (including 5 samples from the UK-NEQAS external quality control scheme). Sera have also been tested with a reference ELISA method (anti-TPO and anti-Tg, BL Diagnostika).
RESULTS/CONCLUSIONS. The new assay uses a calibrator for quantitative determination with average MFI values of 2796 (CV=11.1%) and 1530 (CV=8.9%) for anti-TPO and anti-Tg antibodies respectively (between-run precision, $n=5$). When the quantitative results for the patients were compared between the two methods, the correlation was excellent for anti-TPO ($r=0.96$) and good for anti-Tg ($r=0.91$). The two methods were equivalent in the clinical interpretation of the results ($p=0.13$ for anti-TPO and $p=0.72$ for anti-Tg, McNemar's test). The new assay shows promising characteristics for introduction into the daily routine of a clinical laboratory.

TP3.68

THYROID FUNCTION AND PREVALENCE OF ANTITHYROPEROXIDASE ANTIBODIES (TPOAB) IN AN AREA WITH BORDERLINE SUFFICIENT IODINE INTAKE: EVIDENCE FOR INCREASING THYROID AUTONOMY WITH AGE

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Background: The Nijmegen Biomedical Study (NBS) is a large population-based survey. Randomly selected inhabitants received a postal questionnaire on lifestyle and medical history, which was filled out by 9373 subjects (43%). In 6463 individuals TSH measurements by Immunoluminometric assay (Architect, Abbott), FT4 by Luminescence enzyme immunoassay (Vitros ECI, Ortho) and TPOAb by Fluorescence enzyme immunoassay (AxSYM, Abbott) were performed. Results: Overt thyrotoxicosis was found in 0.4% of the total population and subclinical thyrotoxicosis in 0.8%. Overt hypothyroidism was found in 0.4% and subclinical hypothyroidism in 4.0%. Mean FT4 levels increased with age, both in males and females. The lowest mean FT4 was found among those 30–34 years of age, being 13.0 pmol/L (95% CI 12.8–13.2), while among those older than 85 years mean FT4 was 15.2 pmol/L (95% CI 14.7–15.6). The mean TSH decreased with age in both populations, from 1.46 mU/L (95% CI 1.36–1.57) in the 18–24 year-old to 1.07 mU/L (95% CI 0.92–1.24) in those over 85 years of age. In the total population 8.6% of males and 18.5% of females had positive TPOAb. The mean FT4 level, after correction for age and gender, was 0.50 pmol/L lower in those with positive TPOAb (95% CI 0.32–0.68 pmol/L).
Conclusion: TSH decreases with age while FT4 rises, possibly due to development of mild thyroid autonomy, following longstanding borderline sufficient iodine intake.
Financial support: Abbott Laboratories and Ortho Diagnostics.

TP3.69

NEONATAL THYROTOXICOSIS

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Neonatal hyperthyroidism is rare (1/4000 to 1/50,000 births) but associated with high mortality (12–20%) and possible neurodevelopmental delay in survivors. It is due to transplacental passage of TSH receptor antibodies (TRAb) in mothers with a history of Graves' disease. At highest risk are babies whose mothers have active thyrotoxicosis requiring treatment during pregnancy or previous RAI ablation. Affected mother and baby pairs can be ascertained by measuring TRAb in maternal serum or cord blood. Neonatal thyrotoxicosis may be apparent at birth or delayed for several days and is usually transient, depending on the persistence of maternal TRAb.
A female infant was delivered at term weighing 3.35 kg. On day 2 she was admitted to NICU with tachypnoea (80 min−1) and tachycardia (175–200 min−1), also very irritable, sweaty and flushed. A diagnosis of neonatal hyperthyroidism was made (TSH 0.18 mIU/L, fT4>150 pmol/L). Treatment with propanolol and carbimazole was begun following discussion with paediatric endocrinology. Ultrasound examination of the neck and upper mediastinum demonstrated mild goitre without tracheal compression. Therapy was monitored symptomatically and biochemically using TRAb titres. By day 45 it had fallen to 5.8 from 19.2 U/ml (0–1.5 U/ml). She is now off all therapy and thriving. Long-term neurodevelopment will be monitored. Her mother developed thyrotoxicosis after the first pregnancy, treated with RAI ablation and L-thyroxine replacement. Early in this pregnancy, maternal TRAb was reported to be high (antenatal care was provided elsewhere—no data available) suggesting an increased risk of fetal and neonatal thyrotoxicosis.
This case illustrates the need to measure TRAb in mothers with previous autoimmune thyroid disease so that neonatal thyrotoxicosis may be anticipated and treated promptly.

TP3.70

DEVELOPMENT AND EVALUATION OF IMMUNOASSAYS FOR THYROID PEROXIDASE AND THYROGLOBULIN ANTIBODIES ON THE ABBOTT ARCHITECT® ANALYZER

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Objective: The purpose of our evaluation was to determine the analytical and clinical performance characteristics of two immunoassays under development for thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) on the Abbott ARCHITECT analyzer. Background: Measurement of TPO-Ab and Tg-Ab have clinical significance in a wide variety of situations. The presence of TPO-Ab is a risk factor for development of future hypothyroidism, miscarriage and postpartum thyroid disease, and increased risk of failure during in vitro fertilization. Measurement of Tg-Ab is important in the treatment of differentiated thyroid carcinoma, and may have application for detecting autoimmune thyroid disease in patients with nodular goiter in iodine deficient areas. The ARCHITECT TPO-Ab and Tg-Ab assays utilize chemiluminescent magnetic microparticle immunoassay (CMIA) technology and two-step assay formats. Both assays are standardized to their respective MRC International Reference Preparations. Methods: Imprecision was measured over a five day period using controls and human serum panels. Analytical sensitivity was determined using the 95% confidence method. Concordance versus the respective AxSYM TPO-Ab or Nichols Advantage Tg-Ab assay was also determined. Results: For TPO-Ab, within-run and total imprecision were ≤5.6% and ≤7.3% CV, respectively. Analytical sensitivity was ≤0.16 IU/mL. Concordance to AxSYM ($n=197$) was 100% co-positivity, 92.2% co-negativity, and 94.9% overall agreement. For Tg-Ab, within-run and total imprecision were ≤3.5% and ≤5.1% CV, respectively. Analytical sensitivity was ≤0.15 IU/mL. Concordance to Nichols Advantage ($n=157$) was 86.2% co-positivity, 93.5% co-negativity, and 90.4% overall agreement. Conclusions: Based on these data, we conclude the ARCHITECT TPO-Ab and Tg-Ab assays in development have good sensitivity and precision, and compare well with currently available tests.

TP3.71

EU-PROJECT G6RD-CT-2001-00587: A METHOD COMPARISON STUDY FOR TOTAL THYROXINE IN HUMAN SERUM

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After the successful establishment of a reference measurement system for serum total thyroxine (TT4), the utility of serum-based reference materials to demonstrate metrological traceability of immunoassays was studied. Worldwide, diagnostics companies were invited for a method comparison study (split-sample measurements) with human sera certified by 4 candidate reference measurement procedures (cRMPs) (concentration range: 64 to 269 nmol/L). Results were evaluated by statistical and graphical techniques. The regression equation parameters (ordinary least square) allowed interpretation of the traceability of calibration. The latter was in average acceptable (slope 0.972 ± 0.013; intercept 3 ± 1.2 nmol/L), however, at the individual level, discrepant proportional and/or constant systematic errors were revealed (range of slopes/intercepts: 0.662 to 1.309/-16.4 to 15.2 nmol/L). The absolute difference plots, including the regression lines of the differences, confirmed that some immunoassays showed concentration-dependent systematic errors. For interpretation of the traceability of measurement at the level of the individual sample (accuracy), the correlation coefficient and residuals were used. Again, quite some difference between immunoassays was observed (e.g., range of correlation coefficients: 0.808 to 0.991). This was confirmed from the residuals plots including total error limits.
In conclusion, the study showed that certified panels of sera serve the purpose of demonstrating metrological traceability of TT4 routine immunoassays. Although the split-sample measurements showed in average good comparability with the cRMPs, the results at the individual level revealed difference in analytical validity.

TP3.72

EU PROJECT G6RD-CT-2001-00587: REPORT ON THE ESTABLISHMENT OF A REFERENCE MEASUREMENT SYSTEM FOR TOTAL THYROXINE IN HUMAN SERUM

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The objective of the EU project was to study the feasibility of establishing a reference measurement system (RMS) for serum total thyroxine (TT4). RMSs are needed by the diagnostics industry to meet the metrological traceability demand by the Directive 98/79/EC. Four candidate reference measurement laboratories (cRML) developed a candidate reference measurement procedure (cRMP) based on isotope dilution-liquid chromatography/mass spectrometry and calibrated with a common T4 primary calibrator. After internal evaluation of each cRMP for sufficient precision (within-run and total CV ≤ 1.5 and 2%, respectively), external validation was done in intercomparison studies using common samples (lyophilized and frozen native sera). These intercomparisons showed that all cRMLs performed the cRMPs with fulfillment of predefined specifications: a total and between-laboratory CV of ≤2.0% and 2.5%, respectively and a systematic deviation of ≤0.9%, estimated with a target assigned

from the mean of means by the cRMLs. The mean expanded uncertainty for value assignment to the native sera amounted to 2.1%. In conclusion, a model RMS for serum TT4 is available, comprising a network of cRMLs with externally proven competence to properly perform cRMPs. Admittance of new network members to increase the RML capacity will be done on the basis of the predefined performance specifications. The next task will consist of making the diagnostics industry acquainted with the RMS, e.g., by organizing split-sample measurements with the available certified serum panel.

TP3.73

PERFORMANCE EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF TSH (THIRD GENERATION) ON THE OLYMPUS AU3000I® ANALYZER

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Determination of TSH is used as an aid in the assessment of thyroid status, diagnosis and treatment of thyroid disease.
The Olympus TSH (third generation) assay is a magnetic particle, chemiluminescent immunoassay for the quantitative determination of TSH levels in human serum using the OLYMPUS AU3000i®.
We report here results from our development and evaluation of this automated assay for TSH on the Olympus AU3000i® Analyzer.
Lowest detectable level was determined to be 0.003 μIU/ml. Functional sensitivity was 0.0142 μIU/ml. Assay imprecision was characterized over 20 days (80 reps/instrument) according to NCCLS guidelines. Within run coefficient of variation (CV) for Low, Medium, and High human serum pools (0.1, 1.6 and 37.5 μIU/ml) ranged from 1.5 to 6.9%. Between run coefficients of variation ranged from 5.1% to 8.9%.
The AU3000i TSH assay is specific for TSH demonstrating no significant cross-reactivity with LH, FSH and hCG.
No significant interference was detected from bilirubin, haemolysate and Intralipid. Reagents were optimised to avoid RF and HAMA interference.
Based on our evaluation, we conclude that the Olympus AU3000i® TSH (third generation) assay is a highly sensitive, precise and specific method which meets the requirements for measuring TSH levels in human serum.

TP3.74

PREGNANCY ASSOCIATED PLASMA PROTEIN-A EVALUATION OF A NEW BIOMARKER IN HYPOTHYROIDSM

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Thyroid hormones play a fundamental role in the initiation and maintenance of somatic growth in human. Somatotroph axis includes growth hormone (GH), Insulin like growth factor (IGF-I) and IGF-II, type 1 and type 2 IGF receptors, a family of at least six high-affinity IGF binding proteins (IGFBPs) that determine the bioavailability of IGFs, and a group of IGFBP proteases that cleave IGFBPs that modulate the bioavailability of IGFs. IGFBP-4 protease has been purified from human fibroblasts and identified as pregnancy associated plasma protein A (PAPP-A). Since, hypothyroid patients have low plasma levels of IGF-I and reduced IGF bioactivity; we aimed to investigate the role of PAPP-A, as the IGF-dependent IGFBP-4 protease, in these patients.
Blood samples were collected from 26 hypothyroid patients and from 20 control subjects. Serum PAPP-A was determined by an ultra sensitive enzyme-linked immunosorbent assay (ELISA) and TSH, T3, T4, fT3 and fT4 were measured by chemiluminescent immunometric methods.
Serum PAPP-A levels of hypothyroid patients (5.5 ± 2.7) were higher than control group (4.92 ± 2.1), but this difference was not statistically significant ($p > 0.05$).
The data obtained from this study showed that PAPP-A has no significant function in hypothyroid patients.

TP3.75

PERFORMANCE EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETERMINATION OF TOTAL β-hCG ON THE OLYMPUS AU3000i® ANALYZER

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Determination of Total β-hCG can be used for the early detection and monitoring of pregnancy, and pregnancy related disorders.
The Olympus total β-hCG assay is a magnetic particle, chemiluminescent immunoassay for the quantitative determination of total β-hCG levels in human serum using the OLYMPUS AU3000i®.
We report here results from our development and evaluation of this automated assay for Total β-hCG on the Olympus AU3000i® Analyzer.
Lowest detectable level was determined to be 0.02 mIU/ml.
Assay imprecision was characterized over 20 days (80 reps/instrument) according to NCCLS guidelines. Within run coefficient of variation (CV) for Low, Medium, and High human serum pools (5.4, 21.8, and 553 mIU/ml) ranged from 1.8 to 2.6%. Between run coefficients of variation ranged from 2.9 to 4.3%.
The AU3000i total hCG assay is specific for β-hCG recognising all hCG forms without cross-reactivity to LH, FSH, TSH.
The mean recovery for samples tested for linearity ranged from 95.6% to 106.9%.
No significant interference was detected from bilirubin, haemolysate and Intralipid. Reagents were optimised to avoid RF and HAMA interference.
Based on our evaluation, we conclude that the Olympus AU3000i® Total β-hCG assay is a sensitive, precise and specific method for measuring Total β-hCG levels in human serum.

TP3.76

VARIATION OF THYROID FUNCTION TESTS IN NORMAL PREGNANCY

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Pregnancy is associated with profound physiological changes including thyroid function. It is therefore usual to encounter difficulties in the interpretation of thyroid tests carried out for pregnant women. The aim of the study was to understand the modification in thyroid function during pregnancy that allows for a correct interpretation of thyroid tests.

Serum thyroid stimulating hormone (TSH) and free thyroxin (FT4) were performed in 1049 healthy pregnant Tunisian women with no prior obstetrical complications, using the respective Abbott AxSYM assay. Pregnant women were divided regarding to the trimester of gestation in 284 at the first trimester (T1), 368 at the second trimester (T2) and 397 at the third trimester (T3). Seventy age-matched healthy non-pregnant women (NP) were used as control group. Women with positive thyroid peroxidase antibodies were excluded. Comparisons of means between groups were performed by t test.

Serum FT4 concentrations was significantly lower in T1 (10.65 ± 2.16 ng/l) than NP (12.03 ± 1.78 ng/l) and in T2 (9.98 ± 1.89 ng/l) than T1. No significant differences were observed between T3 (9.97 ± 2.08 ng/l) and T2. Serum TSH concentrations did not vary at the first trimester of pregnancy comparing to the NP women (1.56 ± 0.67 mIU/l). But, TSH levels significantly increased between T1 (1.63 ± 0.94 mIU/l) and T2 (1.91 ± 0.92 mIU/l) and between T2 and T3 (2.12 ± 0.93 mIU/l). Serum FT4 concentration decreases, whereas serum TSH concentration increases through pregnancy. These variations should be taken into consideration for a correct interpretation of thyroid tests in pregnancy.

TP3.77

ASSESSMENT OF GFR IN THYROID DISEASE

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Previous studies have suggested that in patients with untreated thyroid diseases, serum creatinine and cystatin C change in opposite direction. In order to verify this observation we measured the serum concentrations of creatinine and cystatin C in untreated hyperthyroid and hypothyroid patients, treated hyperthyroid and hypothyroid and healthy subjects.

Blood samples were obtained from a total of 179 subjects (36 hyperthyroid, 37 hypothyroid, 30 treated-hyperthyroid, 46 treated-hypothyroid and 30 healthy subjects) and creatinine (using an automated kinetic Jaffe assay) and cystatin C [using particle enhanced nephelometry (PENIA)] concentrations were measured.

Compared to the healthy subjects, serum creatinine concentrations were 17% lower in hyperthyroid subjects ($p=0.004$) and 20% higher in hypothyroid subjects ($p=0.002$). In both treated groups the serum concentrations of creatinine were not significantly different from the healthy subjects (treated-hyperthyroid, $p=0.695$; treated-hypothyroid, $p=0.686$). Compared to the healthy subjects concentration of cystatin C was 21% higher in the hyperthyroid subjects ($p=0.002$). However there was no significant difference in cystatin C between the hypothyroid subjects and the euthyroid group ($p=0.920$). Furthermore the concentrations of cystatin C remained significantly lower in the treated-hyperthyroid group ($p=0.011$) while there was no significant difference between the treated-hypothyroid and healthy groups ($p=0.762$).

We conclude that serum creatinine concentration was higher in hypothyroidism and lower in hyperthyroidism, while cystatin C was higher in the hyperthyroid group. Significantly lowered concentrations of cystatin C in the treated hyperthyroid group could be explained by the possible over-treatment of the hyperthyroid patients and the longer time taken to normalise cystatin C concentrations than thyroid hormone concentrations.

TP3.78

THYROID DISORDERS IN PREGNANCY: PREVALENCE AND ASSOCIATION WITH OBSTETRICAL COMPLICATIONS

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Pregnancy is associated with profound alteration in thyroidal economy resulting from a complex combination of factors specific for the pregnant state. The aims of the study were to assess the prevalence of thyroid disorders during pregnancy and to test the association between thyroid disorders and medical and obstetrical history.

Thyroid stimulating hormone (TSH), free thyroxin (FT4) and thyroid peroxidase antibodies (TPO-Ab) were performed in 1548 consecutive pregnant Tunisian women using the respective Abbott AxSYM assay. Thyroid disorder was defined as TSH>4.5 mIU/L, TSH<0.1 mIU/L and/or TPO-Ab>12 UI/mL.

Thyroid disorder was observed in 159 pregnant women (10.3%). Fifty women have hypothyroidism (TSH>4.5), 25 have hyperthyroidism (TSH<0.1) and 84 have autoimmune thyroiditis (TPO-Ab>12). The prevalence of thyroid disorders did not vary regarding to maternal age, history of obstetrical complications, and personal or familial history of diabetes mellitus. However, thyroid disorders were significantly more frequent in women with autoimmune disease (50% versus 9.6%; $p<0.02$). A history of obstetrical complications (recurrent abortion, foetal death and/or pre eclampsia) was significantly more prevalent in women with positive TPO-Ab than in those with negative TPO-Ab (24.3% versus 16.9%; $p<0.05$).

Pregnancy is associated with a high prevalence of thyroid disorders. Pregnant women with autoimmune disease have a high risk for

thyroid disorders. Positive TPO-Ab is associated with a significant risk of obstetrical complications.

TP3.79

A REVIEW OF THE USE OF TPO ANTIBODY TESTING IN GENERAL PRACTICE

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During a six month period, our review revealed that 57% of 1440 TPO antibody tests undertaken were performed on GP samples.
The majority of these requests were generated within the laboratory in response to a raised TSH (>5mU/L). 73% of this GP cohort had a TSH result of between 5 and 10 mU/L. Of these, 129 patients were TPO antibody positive and 92 TPO antibody negative.
The usefulness of TPO antibody analyses was assessed by a questionnaire requesting information from GPs on individual patient management and outcome.
Of 100 questionnaires issued, 79 were returned, the high response rate possibly reflecting GP uncertainty in this area.
Of 47 patients considered to be clinically hypothyroid, 29 had been started on thyroxine, 25 of whom were TPO antibody positive and 4 negative. The TPO antibody status was defined in 17 of the 18 untreated patients (10 negative, 7 positive).
18 patients were considered to be euthyroid, 15 of whom were not treated. Of these 15, TPO antibody status was defined in 14 (9 negative, 5 positive). The remaining 3 euthyroid patients were TPO antibody positive and were started on thyroxine.
The decision to initiate treatment was taken on the basis of both the TFTs and TPO antibody results for 71% of patients and on the basis of TFTs alone for 21%.
Of those patients started on thyroxine, 88% improved clinically with the remainder reporting no change.
Those untreated, antibody negative patients were typically followed up on an annual basis and those untreated, antibody positive patients quarterly or bi-annually.
It would appear from these data the TPO antibody result did influence patient management.

TP3.80

MACROPROLACTINAEMIA: A CASE REPORT

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A 30 year old female with a 3 year history of secondary infertility who had previously been successfully treated for primary infertility was referred by her family Dr to Obstetrics and Gynaecology for further investigation.
In 2000 she successfully had her first child after treatment with Clomiphene. Since then she has had normal menstrual cycles with no dysmenorrheoa.
Previous medical history indicated that she was a known hyperprolactinaemic patient with a normal pituitary fossa (by MRI in 1998). She was prescribed Cabergoline and compliant with therapy until recently when she decided to stop her medication herself.
In May 2004 she had a prolactin concentration of 30 mU/L (Roche Modular). On the 6th of December a prolactin of 17,804 mU/L was measured. PEG precipitation showed a 3.2% recovery indicating >90% macroprolactin with a calculated monomeric prolactin of 570 mU/L. At this time the patient had no other symptoms and was otherwise fit and well.
Analysis of prolactin by the Bayer Centaur was 1353 mU/L. After PEG precipitation the calculated monomeric prolactin was 486 mU/L.
Analysis of prolactin by the DELFIA method was 3710 mU/L. With a calculated monomeric prolactin of 337 mU/L.
Gel filtration showed 94% macroprolactin with monomeric prolactin of 217 mU/L.
We are not aware of any reports of macroprolactins of this magnitude. The results illustrate the importance of performing PEG precipitation on raised prolactins regardless of the method used.

TP4: HAEMATOLOGY

TP4.01 SELECTED FOR POSTER CLINIC

ELECTROSPRAY MASS SPECTROMETRY (EMS): AN ADJUNCT TO ROUTINE HAEMOGLOBINOPATHY SCREENING

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Screening for Haemoglobinopathies aims to identify variants with potential clinically significant abnormalities, homozygotes or compound heterozygotes, who will benefit from haematological surveillance. Over 850 different mutations in one of the 6 inherited genes which are responsible for coding for the globin chains that form the heterodimers of adult Haemoglobin have been described. Increasing samples are referred to reference laboratories as a result of abnormalities observed on routine HbA1c analysis. These are generally clinically insignificant abnormalities but might compromise diabetic care.
Reference laboratories have a battery of tests to identify these abnormalities including ion-exchange chromatography, isoelectric focussing and as well as the older methods of alkaline cellulose acetate and acid electrophoresis. In the majority of cases these tests will easily identify the variant, however there are a number of haemoglobins both single and multiple gene mutations that remain difficult to identify using established techniques. We have employed EMS to generate $m/z+$ (980–1400) spectra and reconstructed the protein chains using a Bayesian Protein reconstruction programme. This can identify the molecular weights of alpha (normal 15126) and beta chains (normal 15867) and identify the presence of variant chains with a mass difference of >10 Da. Rare abnormalities and multiple mutations

in the same patients have been constructed as a reference library of EMS derived globin patterns. Unstable haemoglobins are particularly amenable to this technique. It has not been possible to identify the fusion chains.
The information gained from the mass of the globin chains in variant haemoglobins has provided useful additional information to simplify the screening process. It is simple and cheap, but will need further development to elucidate same mass substitutions

TP4.02 SELECTED FOR POSTER CLINIC

SERUM FREE LIGHT CHAINS IN SOLITARY BONE PLASMACYTOMA

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Solitary bone plasmacytoma (SBP) is a single bone lesion from a monoclonal plasma cell infiltrate with no evidence of Multiple Myeloma (MM) elsewhere. Standard treatment is local radiotherapy (RT). Patients may experience recurrence, develop MM, or remain free of disease. No prognostic factor has yet been identified and the 10-year disease-free survival is approximately 30%. We measured the serum free light chains (sFLC) in 13 patients with SBP at diagnosis, and where appropriate, during the disease course, treatment and progression to MM. SFLC levels were compared with 8 MM patients diagnosed with a plasmacytoma lesion. The median sFLC level was 32.4 mg/L (range: 13.4–1371.5) for SBP patients and 24.9 mg/L (range: 9.6–527.5) for MM patients. One of the two nonsecretory SBP patients was detected by sFLC assay. Following treatment, sFLC levels decreased in 7 patients (median 61%-range, 6–98). Five of these were in plateau (median overall survival 80 months-range, 12–119) and the remaining 2 patients evolved towards MM at 68 and 98 months. SFLC levels were unchanged in 3 patients and they progressed with a median of 31 months (range, 9–27). Of 3 untreated patients; 2 remained stable and one had an increase in sFLC levels. In an additional kappa light chain plasmacytoma patient, SFLC levels were abnormal at relapse despite unremarkable electrophoreses and MRI. Relapse was confirmed 6 months later by MRI and biopsy despite normal serum and urine electrophoresis. This patient continues to be monitored using sFLC assays during treatment for eventual MM relapse. These data show that sFLC measurements can be used to detect and monitor patients with SBP and may provide a more accurate marker of response after RT.

TP4.03 SELECTED FOR POSTER CLINIC

DIRECT AND INDIRECT FLUORESCENT LABELLING FOR THE DETECTION OF ZAP-70 PROTEIN IN CLL PATIENTS

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CLL is a heterogeneous group of diseases regarding therapy response and survival time. Previously clinical course was predicted based on the identification of chromosomal abnormalities and on the assessment of some serum proteins. More recently CLL patients were divided into 2 groups based on the presence or absence of somatic mutations in the immunoglobulin heavy chain (IgH) and several studies have identified a strong correlation between the unmutated IgH gene and the presence of an intracytoplasmic tyrosine kinase, the Zap-70. This enables us to replace a time and labour consuming sequencing technique with the detection of intracellular Zap-70 in B cells. We have used two and three colour flow cytometric labelling for the identification of this tyrosine kinase, via two different methods. By using a directly PE conjugated antibody (clone 1E7.2, Caltag Laboratories) it was found that 89% of CLL samples displayed Zap-70 values above the 20% cut-off value. By the indirect labelling method utilising the unlabelled 2F3.2 clone (Upstate Biotechnology) Zap-70 positivity was established based on CD3/CD56 negativity. Using this approach 38% of the investigated CLL samples displayed values above 20%. CD38 labelling showed only a partial coexpression with Zap-70 positivity in both cases. Variance in Zap-70 detection is especially prone to differences in staining techniques. Further studies are needed to establish the appropriate cut-off values for the different labelling protocols.

TP4.04 SELECTED FOR POSTER CLINIC

WT1 GENE OVEREXPRESSION AS PROGNOSTIC AND MINIMAL RESIDUAL DISEASE MARKER OF AML PATIENTS

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About 40% of acute myelogenous leukaemia patients lack a suitable specific gene marker for the determination of minimal residual disease (MRD) by a sensitive PCR-based method. However, many of those patients show Wilms tumour gene 1 (WT1) overexpression in the malignant cell clone. In this study, we evaluated the prognostic value of WT1 gene expression and the possibility for using it to determine MRD status in AML patients.
WT1 gene expression in bone marrow (BM) of 82 AML patients at diagnosis was retrospectively determined by quantitative PCR from deep-frozen BM mononuclear cells. Simultaneously, the expression of translocations $t(15;17)$, $t(8;21)$, $t(9;22)$, and inv(16) and mutations in FLT3 gene were tested as candidates for MRD analysis.
Fifty-nine AML patients out of 82 (72%) showed high WT1 gene expression in BM mononuclear cells at diagnosis (WT1 expression >100X BM cell background or >1000X blood cell background). This group included 34 patients (41% of all patients) without specific PCR-based molecular markers for MRD determination. Comparative WT1 and PML-RARA gene expression patterns were found during successive remission and relapse phases in a patient with $t(15;17)$.
The overall survival at two years was 43% in WT1+($n=42$) and 50% in WT1−($n=20$) patients (not significant). Nor was the disease-free

survival significantly different between the WT1 − (n = 15) and WT1 + (n = 35) groups. We conclude that WT1 gene expression is high in most AML patients. This would facilitate its use as a sensitive marker for MRD. The prognostic value of WT1 expression at presentation in this cohort of patients was low.

TP4.05 SELECTED FOR POSTER CLINIC

APPEARANCE OF MULTIDRUG RESISTANCE (MDR) AS AN EARLY PREDICTOR OF RELAPSE IN IMATINIB TREATED CHRONIC MYELOID LEUKAEMIA PATIENTS

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Background: The MDR-1 gene product P-glycoprotein mediated multidrug resistance is thought to play a major role in the outcome of chemotherapy. Multidrug resistance is a major problem during the treatment of various haematological malignancies including chronic myeloid leukaemia (CML). It is known that the C3435T polymorphism of the mdr-1 gene has significant effect on the level of mdr-1 gene expression. In addition, the measure of bcr-abl gene expression is proportional to the severity of the disease in CML. In our study we compared MDR1 properties with the bcr-abl gene expression levels.
Materials and methods: In addition to cell morphology, cytochemistry and flow cytometric detection of the phenotype our investigation focused on the parameters of the mdr-1 gene and bcr-abl gene. Real time RT PCR technique provided a quantitative assay to monitor the expression of these genes (mRNA quantification) and to determine the mdr-1 genotype.
Results: MDR-1 genotype, mdr-1 mRNA and bcr-abl mRNA expressions were followed up sequentially during a period of 2 to 3 years of clinical treatment in 17 imatinib (Gleevec) treated CML patients. Notably, persistant Philadelphia positivity was frequently associated with MDR1 gene activation. Moreover, mdr1 mRNA expression levels predicted early acceleration and Philadelphia positive blast crisis.
Conclusion: Revealing of the mdr-1 genotype of the patients and the follow-up of the mdr-1 and bcr-abl gene expressions could improve the efficiency of the used therapy and the chemotherapy treatment could be adjusted and modified in due time.

TP4.06 SELECTED FOR POSTER CLINIC

SCREENING FOR MONOCLONAL GAMMOPATHY: IMPROVED SENSITIVITY USING SERUM FREE LIGHT CHAIN ASSAYS

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Screening for monoclonal gammopathy (MG) in the UK commonly consists of serum protein electrophoresis (SPE), serum immunoglobulin (Ig) levels and detection of Bence Jones proteinuria (BJP). This approach detects 96% of clinically significant plasma cell dyscrasias. In conditions such as non-secretory myeloma, light-chain myeloma where the renal threshold for light chain excretion has not been reached; amyloidosis and light-chain deposition disease may be missed. We aimed to compare the ability of serum free light chain (sFLC) assays, along with routine screening methods, to detect patients with MG. We assessed 217 consecutive samples referred to a district general hospital for investigation of possible monoclonal gammopathy and 24 known cases of multiple myeloma. The 217 samples received routine SPE and total Ig measurement. Immunofixation electrophoresis (IFE) was performed if an abnormality was detected in SPE, Ig levels or if BJP was detected. SFLC were assessed by Freelite™. SPE and sFLC ratios combined detected 24/24 of the known monoclonal gammopathy cases. The remaining 217 samples were normal by SPE, suggesting no new cases of monoclonal gammopathy. However, 8 cases (3.3%) had an abnormal free light chain ratio and available matching urines did not reveal BJP (5/8). IFE in all 8 cases was shown to be normal. 2 cases had hypogammaglobulinaemia. Review of the case-notes confirmed 3 cases of multiple myeloma, 2 requiring treatment. The remaining five cases are under investigation. For known MG, the combination of sFLC and SPE has a higher sensitivity {27/27 cases (100%)} than SPE with BJP {24/27 cases (89%)}. This improved sensitivity has important implications for the detection of clinically important plasma cell dyscrasia.

TP4.07

THE UTILITY OF FOLATE TESTING IN ROUTINE CLINICAL PRACTICE

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Folate levels are routinely ordered in the evaluation of macrocytosis with or without frank anaemia, however the value of this test has recently been questioned.
We evaluated the clinical utility of folate testing in routine clinical practice.
We conducted a retrospective review of all erythrocyte folate assays performed over a one-year period (2002–2003) at Aalborg Hospital in Denmark. We determined the frequency of low values (<350 nmol/L), then reviewed the medical records of all patients with low values to determine whether low folate levels changed clinician behaviour.
The prevalence of low serum folate levels in this clinically suspected population was 2.9% (95 out of 3328 patients). Only 40 of the 95 patients with low serum folate levels were given folate replacement, representing only 1.2% of the clinically suspected and tested patients.
Folate values were rarely low in the population tested, and low values infrequently led to a change in clinician behaviour. The low prevalence can be due to the inappropriate testing of patients without a true indication or the testing of patients already taking folate supplements or on a folate-replete diet.

TP4.08

COMPARISON OF SEVEN COMMERCIAL PROTHROMBIN TIME REAGENTS AND FOUR CALIBRATORS: POOR AGREEMENT BETWEEN INTERNATIONAL NORMALISED RATIO (INR) RESULTS

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Prothrombin time (PT) has long been the most popular test for monitoring oral anticoagulation therapy. The international normalised ratio (INR) was introduced to overcome the problem of marked variation in PT results between different laboratories and the varying recommendations for patient care. According to this principle, all reagents should be calibrated to give identical results and the same patient care globally. This is necessary: (i) in monitoring single patients, and (ii) in application of the results of anticoagulation trials and guidelines to clinical practice.
We took blood samples from 150 patients for whom oral anticoagulation had been prescribed. Plasmas were separated and PTs determined using seven commercial reagents and four calibrators. The agreement in results obtained was assessed using the Bland-Altman strategy: differences in INR values between each possible pair of methods were plotted against their mean INR value.
Serious problems in agreement of the results of these INR methods were revealed in 19 pairs out of a total of 21 comparisons. Clinically acceptable agreement was demonstrable with only two pairs of methods. The problems were (i) a substantial difference in INR values, and (ii) a systematic increase in the difference as well as (iii) a systematic increase in the variation in difference with increasing INR values.
The agreement between commercial INR methods is poor and clinically not acceptable. Calibration strategies have not been successful. This may severely compromise the reliability of these methods both in single patient monitoring as well as in the application of oral anticoagulation guidelines and trial results to clinical practice.

TP4.09

PROTEIN C AND FREE PROTEIN S IN PATIENTS WITH VENOUS THROMBOSIS

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Introduction: Proteins C and S belong to the group of vitamin-K dependent proteins synthesized in the liver. Physiologically, proteins C and S have an essential anticoagulant function. In the activated state protein C regulates the coagulation process in the presence of protein S (cofactor of activated protein C). The deficiencies of protein C or S increase the risk of venous thrombosis.
The aim of our study was to determine the free protein S (active form) and protein C levels in patients with venous thrombosis.
Methods: The study was carried out in 24 patients with venous thrombosis at the age 8–55 years. The plasma's protein C levels were assayed by synthetic chromogenic substrate method; the levels of free protein S were estimated by immunoturbidimetric method using analyser STA Compact (Diagnostica Stago).
Results: The levels of protein C and free protein S were normal in 6 cases, including 4 cases of patients with venous thrombosis at the age 8–14 years (not congenital deficiency). The levels of protein C and free protein S were decreased in 17 cases, in 1 case the level of protein C was normal but the level of free protein S was decreased. The decreased levels of plasma protein C and free protein S were caused by hepatic disorders in 14 cases, during therapy with oral anticoagulant—in 3 cases. The normal protein C and decreased free protein S levels were found during pregnancy.
Conclusion: Venous thrombosis is associated with acquired deficiency of protein C or free protein S. The measurement of protein C and free protein S is useful in diagnosis of thrombotic disease.

TP4.10

DEVELOPMENT OF A PILOT EQAS SCHEME FOR PERIPHERAL BLOOD SMEARS IN AN ESTABLISHED EQAP (PROGRAMA BUENOS AIRES)

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Buenos Aires EQAS has been an established Latin American External Quality Assurance Programme since 1979. A Haematology Section Pilot Scheme for blood smears was developed to validate QC samples. Smears from two EDTA blood samples, normal and pathologic, were manually prepared by the same operator and stained by May Grünwald-Giemsa technique. Both smears were sent to 35 routine and specialized labs.
To perform the internal validation of the samples, three smears were randomly sorted and repeatedly assessed by three different observers during three non-consecutive days.
Statistical analysis: median and quartiles (P25, P75) were considered for leucocytes and platelets; % concordance, for red cells (RC) morphology.
Results: The return rate was 50%.
Normal blood smear: Dispersion of results was similar to that obtained during internal validation. Median and Percentiles (P25, P75): neutrophils 49% (44%, 55%); lymphocytes 38% (33%, 43%) and platelet count 57 per thousand RC (48, 67). RC morphology: 80% concordance on normochromasia.
Pathologic blood smear (patient with autoimmune haemolytic anemia): Dispersion of results for white blood cells was higher than in normal smears. Consensus obtained: >80% for polychromasia, anisocytosis, macrocytes, Howell–Jolly bodies and basophilic stippling; near 50% for poikilocytosis, microcytes, Cabot rings, target cells and teardrop cells.
Presence of erythroblasts was reported in 14/17 returns (range: 224–1022 per 100 WC).

Conclusions: The protocol for blood smears preparation resulted appropriate for use in EQAS. Higher variability was due to low count of minor white cells components and to observer subjectivity for evaluation of RC morphology as laboratories had different training. It will be advisable to categorize labs to analyze results.

TP4.11

DEFECT OF AN AGGREGATION PLATELET FUNCTIONS DURING CHRONIC MYELOPROLIFERATIVE DISEASES

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A study of primary hemostasis was conducted for 84 patients with varying chronic myeloproliferative diseases (MPDs): chronic myeloid leukemia ($n=41$), polycythemia vera ($n=26$), essential thrombocythemia ($n=10$) and idiopathic myelofibrosis ($n=7$). The monitoring group consisted of healthy donors of both sexes comparable by age.

Platelet aggregation was induced with the following inductors: adenosine5′-diphosphate (ADP) in final concentrations 0.5 μM, 1.5 μM, 2.5 μM and 5.0 μM; epinephrine (Adr) -5.0 μM and solution of a collagen 2.0 mg/ml. The platelet count was adjusted to a standard value (in thrombocytosis dilution was made).

For all forms of MPDs there was differential platelet dysfunction characterized by a decrease in aggregation platelet function, down to complete suppression, while in infrequent cases an increase in response to a stimulation by the above listed inductors. It is necessary to note that against a background of considerable suppression of platelet aggregation in response to Adr and ADP, with collagen—the platelet result remained. The aforementioned may be related to presence of defects in the transfer mechanism of a signal for these patients, instead of disturbance in the release reaction. It is possible to suspect, that the detected defect was conditioned by disturbance of calcium mobilization in thrombocytes.

The detected disturbances of thrombocytes functions, probably, are a consequence of platelet origin from a neoplastic tumor megacaryocytes clone. Moreover, this hypothesis is confirmed by absence of similar changes during secondary thrombocytoses, and more importantly, by normalization of platelet function after an allogenic bone marrow transplantation.

TP4.12

SHOULD THE LABORATORY INITIATE REQUESTS FOR MYELOMA SCREENING?

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Whether a laboratory should respond to significantly abnormal results by initiating further investigations is a controversial area. Within Northumbria Healthcare Trust, at the request of the local Haematologists, the Clinical Chemistry Department screens specimens with significantly raised serum globulins (total protein - albumin) in LFT profile for myeloma by protein electrophoresis. The criteria for screening used depended on globulin level and the patient's clinical picture, especially anaemia, renal impairment and bone pain.

At Wansbeck laboratory, in the first year after the introduction of the service, there were 418 (16% of total of 2656 requests) serum protein electrophoreses initiated by the laboratory and 15 patients were newly diagnosed with myeloma. In the following year, with refined criteria, the laboratory initiated 161 (7% of total of 2232) electrophoreses, which detected 9 out of 18 newly diagnosed cases of myeloma (other diagnostic routes: 5 with bone pain of which two were light-chain myeloma and one MGUS on follow-up, 1 incidental X-ray of non-pathological fracture and 3 unknown).

Prior to introducing the screening service the annual incidence of myeloma at Wansbeck Hospital was 4–7 cases pa, similar to that at North Tyneside where a similar screening service has been in operation for about 10 years.

It appears that when a laboratory introduces a myeloma screening service by initiating serum protein electrophoresis on specimens selected by appropriate biochemical and clinical criteria an increased detection rate can be achieved.

TP4.13

VALUE OF SERUM TRANSFERRIN RECEPTOR (STFR) TO TREATMENT OF IRON-DEFICIENCY IN HEMODIALYSIS PATIENTS: A LONGITUDINAL STUDY

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Introduction: Hemodialysis (HD) patients can suffer from an anemia which is iron- and/or hrEPO-dependent. Ferritin (<100 μg/L) and iron saturation (<20%) have been the criteria for supplementation. Recent literature suggests that the sTfR might be superior in assessing the iron status. The value in HD patients remains to be assessed since sTfR is affected by hrEPO. We studied the impact of changes in therapy on the sTfR to assess the value of the sTfR in predicting the iron status.

Study design: In the hemodialysis population (43 females, 51 males), therapy was registered and markers of iron status plus sTfR were measured at two time-points with 3 months interval. On the basis of changes in therapy, 7 groups were analysed for changes in iron status and sTfR.

Results: 37% of all patients had a Hb<7.0 mmol/L and 34% were iron-deficient according to the criteria. 44% of these iron-deficient patients had sTfR>2.08 mg/L; in the non-iron-deficient patients, 9.5% had sTfR>2.08 mg/L. Only one patient had sTfR>4.00 mg/L. Changes in iron-supplementation did not affect the sTfR. Only one subgroup ($n=6$) showed an increase in hrEPO paralleling a rise in Hb (5.6 to 6.7 mmol/L) and a decrease in sTfR (2.35 to 2.00 mg/L).

Conclusions: Although the % of patients with an elevated sTfR is highest among iron-deficient group, sTfR levels are in general low with a small range (1.77 ± 0.66 mg/L). Fine-tuning of therapy did not significantly affect sTfR concentrations. In our HD-patients sTfR is not a useful parameter in assessing the iron status.

TP4.14

AUTOMATED CD61 IMMUNOPLATELET ANALYSIS IN ROUTINE HEMATOLOGY LABORATORY

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Automated hematology analyzers use aperture impedance or optical methods to determine the absolute platelet counts. Non-platelet events (red cell microcytes, schistocytes, WBC fragments, protein aggregates) are known to interfere with platelet counts both by impedance and optical methods. Precise and accurate platelet counts are important for prophylactic platelet transfusion thrombocytopenic patients.
Immunoplatelet (CD61) assay (ABBOTT, CELL-DYN 4000) improves the accuracy of platelet counts and reduces interference by nonplatelet particles.
We used this method in two different cases when was impossible to provide accurate platelet counts with standard methods.
Case 1: child with congenital heart disease
Routine tests: RBC 9.39×10^{12}/L, MCV 59.5 fl, Plti 75.0×10^{9}/L PltClmp, Plto 84.0×10^{9}/L
ImmunoPlt (CD61): Plt CD61 93.3×109/L
Case 2: patient undergoing chemotherapy
Routine tests: Plti no result, Plto 5.95×10^{9}/L
ImmunoPlt (CD61): Plt CD61 3.50×10^{9}/L
The immunoplatelet (CD61) assay utilizes a fluorescent labeled monoclonal antibody specific for platelets. The assay is fully automated, simple to use, accurate. It takes less than 6 min from aspiration to report. This assay is ideal for arbitration purposes in the routine hematology laboratory.

TP4.15

HEPARIN-INDUCED UPREGULATION OF TFPI MRNA EXPRESSION IN HUMAN ENDOTHELIAL CELLS IN VITRO

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Tissue factor pathway inhibitor (TFPI), a multivalent Kunitz-type serine proteinase inhibitor, has been identified as a potent natural anticoagulant which regulates the tissue factor-mediated coagulation. Since it is well known that in vivo the application of heparin leads to a release of TFPI from the vascular endothelium, the aim of this study was to elucidate whether the incubation of the human endothelial cell line Ea.hy 926 with different heparan sulfates leads to an alteration of the TFPI mRNA expression.
Based on the LightCycler® system we established a real-time PCR assay for a specific detection of the TFPI gene and the housekeeping genes. Ea.hy 926 cells were cultured and low-molecular-weight heparin (LMWH), unfractionated heparin (UFH), heparan sulfate and chondroitin sulfate were added. RNA was isolated from samples taken after incubation times of 4, 8 and 24 h and TFPI mRNA expression was quantified relatively to the housekeeping genes.
Incubation with LMWH as well as UFH led to an up to 4-fold upregulation of TFPI mRNA expression, whereas heparan sulfate and chondroitin sulfate did not have an upregulating effect. The strongest effect was detectable after 4 h and decreased after longer incubation periods with higher concentrations of heparin (10 IU/mL) leading to a stronger effect than 1 IU/mL.
In summary we could demonstrate that incubation with LMWH as well as UFH lead to a concentration dependant increase of TFPI mRNA expression in human endothelial cells, whereas HS and CS did not have an upregulating effect. These findings are in concordance with the in vivo effects of heparin.

TP4.16

HB FLORIDA: A NEW ELONGATED C-TERMINAL BETA-GLOBIN VARIANT CAUSING DOMINANT BETA-THALASSEMIA PHENOTYPE

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An elongated C-terminal beta-globin variant, caused by the deletion of one nucleotide (-C) in between codons 140/141 (GCC/CTG→GCC/TG), which modified the C-terminal sequence and added 10 more residues to the beta-chain [(141)Trp-Pro-Thr-Ser-Ile-Thr-Lys-Leu-Ala-Phe-Leu-Leu-Ser-Asn-Phe-(156)Tyr-COOH], was found in an 8-year-old Argentine girl of Spanish descent with a clinical picture of beta-thalassemia intermedia.
The patient presented with chronic moderate hemolytic anemia (RBC=3.8×10^{12}/L, Hb=8.6 g/dL, Hct=28%), with pallor, jaundice and liver and spleen enlargement, having required blood transfusion for 5 times, during viral and bacterial infections. Peripheral blood analysis revealed a remarkable degree of anisocytosis with microcytosis (RDW=28%, MCV=73.0fl), poikilocytosis (with ovalocytes and schistocytes), hypochromia (MCH=22.6 pg), 13% of reticulocytes and 2% of erythroblasts, punctate basophilia, elevated Hb A2 and Hb F levels (5% and 13%, respectively), without any detectable abnormal Hb (Hb and globin chain electrophoreses/HPLC). Tests for unstable hemoglobins were weakly positive, but the staining of the bone marrow cells with methyl violet allowed the visualization of many inclusion bodies in erythroblasts.
These results suggest that this is probably a hyperunstable variant whose proteolysis in the bone marrow precursors results in dominant beta-thalassemia phenotype, since the other beta-locus

showed no alteration. It is also a novel mutation since the mother's carrier was completely normal and the father, although not available for study, had no clinical complaints.
Financial Support: FAPESP and CNPq/Brazil.

TP4.17
EXTERNAL QUALITY CONTROL OF HEMOGLOBINOPATHIES IN THE NETHERLANDS
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Hemoglobinopathies and the associated genetic risk in offspring of carriers are still underestimated in The Netherlands. In 2002, after a general inventory on the diagnostic potential throughout the country, the Dutch Association for Laboratory Hematology (VHL) proposed standard protocols for Hemoglobinopathies Diagnostics.
In parallel to this the Dutch organization for external quality control (SKML) updated the scheme for abnormal hemoglobin detection. Specimens are now fully characterized at hematological, biochemical and molecular level at the Dutch Hemoglobinopathies Reference Laboratory. 40 laboratories, using HPLC, Electrophoresis and Capillary Electrophoresis participate in the scheme. Along with the specimens they are supplied with brief information (ethnic origin, anamnesis, morbidity, blood indices). Participants are asked to report identity and amount of observed variants and to give a putative diagnosis as well as an advice in respect to genetic risk.
Results of the 2004 scheme are presented. Normal specimens are correctly interpreted by 100%. For HbS and HbC related patterns correct percentages are 98% (type AC), 96% (type CC), 96% (type SC), 92% (type C with HbF), 75% (type SE). Beta-thalassemias with elevated levels of HbA2 and HbF are correctly seen by 85% (15% misses HbF). Elevated HbF is seen by 92%. Type DD is found correctly by 55% while 22% reports an "unknown" variant and 18% the wrong type. With HbJ-like specimen 32% reports type J, 63% an "unknown" Hb. It is concluded that detection of HbS, C and F does not cause difficulties but that HbD- and J-specimens are often misinterpreted.

TP4.18
IDENTICAL IMMUNOPHENOTYPIC CHARACTERISTICS OF ACUTE MYELOID LEUKEMIA IN CHILDREN WITH DOWN'S SYNDROME
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It is well known, that children with Down's syndrome have a higher risk of acute leukemia than the remaining pediatric population. During the first 3 years of life acute myeloid leukemia (especially AML M7) predominates.
We analyzed immunophenotype of blast population of 4 children with cytogenetically confirmed Down's syndrome and neoplastic myeloid proliferation (all girls, 12–26 months old). In 3 cases there were more than 20% blasts in bone marrow; in one case number of blasts was below 20% of bone marrow cells. In all cases blasts had specific morphologic features: basophilic cytoplasm with blebs and dense nuclear chromatin. Cytochemical staining for blast myeloperoxidase was negative.
Flow cytometric analysis revealed that in all cases blasts expressed CD45 dim, CD34, CD117 and CD33. Megakaryocytic blast differentiation was suggested by expression of CD41a and CD61 markers. In all cases there was an aberrant expression of the T-lineage-associated antigen CD7 and CD4 and CD56. Blasts were HLA-DR negative.
We concluded that myeloid blasts in children with Down's syndrome present with a specific immunophenotype distinct from AML in general population and is consistent with megakaryocytic origin.

TP4.19
ERYTHROCYTES NON-PROTEIN IRON IN MDS PATIENTS AND PRETERM NEWBORNS
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Iron is released from haemoglobin or its derivatives in a nonprotein-bound form in a number of conditions in which the erythrocytes are subjected to oxidative stress. The released iron could play a central role in generating reactive oxygen species.
We studied the effect of the free radical generating hypoxanthine/xanthine oxidase (Hx/XO) system on erythrocytes from 17 patients with myelodysplastic syndrome (MDS), (untransfused), 19 prematurely newborn infant, and 22 healthy adults volunteers.
Non-protein iron concentrations were measured after 30 min incubation of erythrocyte at 37 °C in buffer Hx/XO system, as iron (III)-bathophenantroline complex with capillary electrophoresis.
In the erythrocytes from the MDS, preterm, and healthy group, the mean values of non-protein iron concentrations (mean ± S.D.) were 6.19 ± 2.591, 7.34 ± 4.941 and 2.90 ± 0.457 nmol/gHb, respectively. The experimental results indicated that the Hx/XO system caused an increase of erythrocyte non-protein bound iron, (for MDS group, preterm infant, and healthy group: 90.33 ± 48.02, 109.98 ± 61.83, and 54.58 ± 23.14 nmol/gHb. respectively), that is not released into the extracellular space. The addition of iron ions to Hx/XO system intensifies the damages on erythrocyte membrane, and iron ions gain access to the extracellular space in concentrations approximately 10 times the iron concentration.
The results indicate that the erythrocytes of the three groups, exposed to the Hx/XO system used in our experiments, differ in their response to the formed free radicals probably because of the already present non-protein bound iron, exhaustion of antioxidant defence and also possibly some changes in membrane lipid structure.

TP4.20

CORRELATION OF CYTOGENETIC ABERRATIONS WITH IMMUNOPHENOTYPING DATA IN ADULT ACUTE B-LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is a heterogeneous disease with clinically, morphologically and prognostic distinct subset. Data from immunophenotyping and molecular-cytogenetic analyses allow better understanding of this complex disease entity. The aim of our study is to determinate molecular-cytogenetic aberrations in adult B-ALL and their correlation with immunological subgroups of disease. We carried out conventional cytogenetic and FISH analyses in 20 adult patients with newly diagnosed acute B-ALL and subdivided them to 4 immunological B-ALL variants (according to European group of immunological characterization of leukemias-EGIL)—pro-B (7 out of 20, 35%), common B (6 cases, 30%), pre-B (3 cases, 15%) and mature B-cell (4 cases, 20%). Frequency of molecular-cytogenetic aberrations was—*t*(9;22)–10%, *t*(4;11)–5%, *t*(8;14)–20%, del12p–5%, del4p–10%, *i*(7q)–5%, *t*(1;19)–5%, complex karyotype–10%, normal karyotype–15%, hyperdiploidy–10%, trizomy 8–5%. The most frequent immunophenotype was pro-B cell ALL with poor prognostic cytogenetic markers-bcr/abl fusion, MLL rearrangements, del12p and trisomy 21. CD10(+)ALLs are frequently associated with hyperdiplody and *t*(9;22), but we found low incidence of *t*(9;22) without ploidy changes. *T*(1;19) is typical for childhood pre-B ALL, but it is an unusual aberration in adult CD10+ ALL. We distinguished a relatively high incidence (20%) of B-ALL which associated mature B-phenotype and *t*(8;14) (q24;q32)/C-MYC (+) (Burkitt's type leukemia). Conclusion: in this study we found association of cytogenetic aberrations with immunophenotypes only in mature B-cell leukemia. The other immunophenotypes are characterized with genetic heterogeneity and the presence of unusual for adult ALL cytogenetic abnormalities—*t*(1;19) (q23;p13) and trisomy 8.

TP4.21

IMMUNE PHENOTYPING OF ACUTE LEUKEMIA IN CHILDREN IN THE REPUBLIC OF BASHKORTOSTAN, RUSSIA

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Acute leukemia (AL) is one of most common oncological disorders in childhood, with an incidence in the Republic of Bashkortostan, Russia, of 3.0 in 100,000 children. Immune phenotyping of bone narrow blast cells by specific monoclonal antibodies helps to determine the line-associated and stage-specific surface CD antigens, identify the immune variant of leukemia, and in most cases verify the T- or B-line generation of blasts and the stage of its differentiation arrest.

The basic aim of this study was to characterize the leukemia cells and verify the variant of AL in patients treated at Republic Hospital for Children, Ufa, Russia. Investigations of 75 children of 0 to 14 years of age were performed in 2000–2003 using indirect fluorescent assay on LEITZ microscope and flow cytofluorometry on a FACS Calibur (Becton Dickinson) using panels of monoclonal antibodies from MedBioSpector (Russia) and Becton Dickinson (USA) for determination of surface and cytosol HLA-DR, CD34, CD1, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD21, CD22, TdT, CD79a, IgM, CD13, CD14, CD15, CD33, cytMPO, CD41, and CD61.

As a result of investigations performed acute myeloblastic leukemia (AML) was diagnosed in 21.3% patients, different variants of acute lymphoblastic leukemia (ALL) – in 78.7%. The B-blast cells were determined in 66% cases, T-line blasts – in 34%. In T-lineage leukemia the pro-T-ALL (T-I variant) was observed in 3 cases, pre-T-ALL (T-II) in 7 cases, cortical T-ALL (T-III) in 5 cases, and T-mature ALL (T-IV) in 5 cases. In B-lineage leukemia the B(common)-ALL (B-II) was determined in 23 children, pre-B-ALL (B-III) in 8 children, B-mature ALL (B-IV) in 2 children, and pre-pre-B-ALL (B-I) in 6 children. We have found that the prognosis of ALL in children is poorer if the expression of CD34+ and CD5+ is observed in more than 50% of blasts. We believe these data are useful in choosing adequate treatment for improved response.

TP4.22

COMPARISON OF QUICK AND OWREN PROTHROMBIN TIME LABORATORY METHODS

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Prothrombin time (PT) is the most commonly used coagulation test in routine laboratories and is used for monitoring oral anti-coagulation therapy, prevention and treatment of venous thromboembolism, artrial fibrillation and other indications.

The aim of this study was to compare two major PT methods (Quick and Owren, combined thromboplastin reagent) for INR measurement.

We measured PT with an ACL 7000 analyser. We used three Owren and three Quick reagents for PT and estimated ISI for each reagent using two local and two manufactured ISI calibrator sets. Coagulation time was measured using five different normal plasmas to assess variation for every reagent and both methods. We studied the analytical bias for every reagent and both methods at INR 1.0 and 2.5.

The mean CV % of Owren reagent ISI was 2.40% and Quick reagent ISI was 12.85%.

The mean CV % of normal plasma seconds for the Owren method was 2.54% and for Quick 4.02%. Absolute error was at INR 1.0 and INR 2.5 for Owren 0.00 and 0.04 INR and for Quick 0.01 and 0.16 INR.

In the comparison the Owren method gave better results than the Quick method in ISI calibration, normal plasma variation, within-run analytical variation and absolute error at 2.5 INR. The INR system is more demanding on analytical quality than earlier units

(*R*, %). The Owren PT offers advantages over the Quick PT in harmonisation of the INR system.

TP4.23

SCREENING FOR SUBCLINICAL HYPOTHYROIDISM IN A POPULATION OF PREGNANT WOMEN IN LITHUANIA

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The thyroid function tests screening is considered to be useful for many types of patients, especially for pregnant women. Recent studies suggest existing association between pregnancy and the thyroid dysfunction. Maternal hypothyroxinaemia may effect neuropsychological development and cognitive development of the foetus. Fertility problems and miscarriages are observed among women with subclinical hypothyroidism and positive test for anti-TPO Ab. If the mild thyroid function abnormalities during pregnancy are present, they may be linked to impairment of the child born from that pregnancy.

The aim of the study was to evaluate usefulness of thyroid function tests screening in pregnant women.

100 blood samples from pregnant women 15–18 gestational weeks admitted to the Centre of Medical Genetics of Vilnius University Hospital Santariskiu Clinics were selected for the study. Whole blood samples were collected into sample tubes (Vacutainer, BD). TSH, Free T4, Total T4, Total T3, Free T3 and Anti-TPO Ab tests were measured on IMMULITE 2000 and IMMULITE (DPC, USA) by chemiluminescent enzyme-labelled immunometric assays.

The mean values of TSH-1.03 μU/ml, FT4-1.15 ng/ml, T4-9.78 μg/dl, T3-183.57 ng/dl, FT3-3.52 pg/ml, Anti-TPO Ab-37.32 IU/ml were obtained. In 12 cases anti-TPO Ab values were significantly higher with mean value of 261.96 IU/ml in comparison to whole group mean value. In 15 cases FT3 values exceeded normal range (1.5–4.1 pg/ml) with the mean value 4.61 pg/ml.

We found different type of thyroid abnormalities in 27% pregnant women that is why screening of thyroid function tests in young women population and during pregnancy could be useful to avoid possible problems associated with foetus development.

TP4.24

THREE NOVEL ALPHA-GLOBIN VARIANTS: HB ITAPIRA [alpha-30(B11)GLU→VAL(alpha 1)], HB BOM JESUS DA LAPA [alpha-30(B11)GLU→ALA(alpha 1)] AND HB BOA ESPERANCA [alpha-16(A14) LYS→THR (alpha2)]

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Three new hemoglobin variants were found in four Brazilian blood donors.

Hemoglobin Itapira was detected in a Caucasian man from the Southeastern region; electrophoresis at alkaline pH showed an HbS-like band, not distinguished from HbA at acidic pH. Cation-exchange HPLC exhibited a short peak (5.5%) at D-Window. The abnormal alpha-chain migrated slower in globin-chain electrophoresis (acidic pH). DNA sequencing revealed a GAG→GTG (Glu→Val) substitution at codon 30 of alpha1-gene, in heterozygosis, in the patient, his mother and brother. They were also heterozygous for the alpha-alpha-alpha-anti3.7 haplotype, explaining the low percentage of the variant and suggesting association.

Hemoglobin Bom Jesus da Lapa also results from a mutation at codon 30 of alpha1-gene (GAG→GCG; Glu→Ala). It was detected in a woman of African descent from the northeastern region; Hb electrophoresis was similar to that of Hb Itapira, but it eluted with HbA2 on HPLC (24.8%). Globin-chain electrophoresis was normal.

Hemoglobin Boa Esperanca was detected in two unrelated individuals, one of African descent, both originating from Southeastern Brazil. It moved faster than HbA at alkaline pH, was not distinguished at acidic pH, and eluted as an additional peak at P3 on HPLC (25%); globin electrophoresis showed a slower alpha-chain. Sequencing revealed a base substitution at codon 16 of alpha2-gene, in heterozygosis (AAG→ACG; Lys→Thr). Beta-cluster haplotypes were suggestive of a common ancestor.

All the three variants described here showed normal instability and solubility tests, suggesting that their residue replacements did not affect the structural stability of the Hb molecule.

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TP4.25

SERUM FOLATE AND B12 ON COURSE OF PARENTERAL NUTRITION

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Patients receiving parenteral nutrition especially in a hypercatabolic state related to the acute phase reaction are likely to develop various types of nutritional deficiency including, vitamins and trace elements. The aim of the study was to compare serum folate and vitamin B12 in 46 nonseptic and 49 septic patients on the same regime of parenteral nutrition containing vitamins for over 3 weeks. Groups were divided according to serum CRP value: <20 mg/l nonseptic, >21 mg/l septic group. Blood was collected in the morning. Serum folate and B12 were measured by chemiluminescence on the ACS Bayer, CRP by immunoturbidimetry on the Cobas Integra Roche. Mean B12 values were 580 and 615 pg/ml, respectively, and were not significantly different. Serum folate was significantly lower in septic patients (6.7 compared to 10.2 ng/ml). No correlation was found between CRP and either folate or B12. Lower folate concentration may be the result of intensive utilization of folate to form nuclei of leucocytes as the WBC count was elevated in the septic group.

TP4.26

CLINICAL EFFICIENCY OF THE iQ®200 COMPARED WITH HEMACYTOMETER CELL COUNTS FOR SEROUS FLUID

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Objective: To determine the clinical efficiency of the iQ®200 Automated Urine Microscopy Analyzer Body Fluids Module (iQ200) in determining concentrations of RBC and WBC in serous fluids in comparison with traditional hemacytometer counts.
Relevance: Hemacytometer cell counting requires time and skill. Several blood counters FDA cleared for body fluid analysis have common minimum reportable concentrations of 10,000/microliter and 50/microliter for RBC and WBC, respectively. The iQ200 allows identification and quantitation of RBC and WBC even when their concentrations differ significantly.
Methods: Ninety-five serous fluid specimens were analyzed by both methods. Fifty-eight percent had RBC concentrations <10,000/microliter and 17% had WBC concentrations <50/microliter. RBC concentrations ranged from 1 to 1,430,000/microliter with a median of 6825/microliter. WBC concentrations ranged from 1 to 23,150/microliter with a median of 625/microliter. Hemacytometer counts were performed on the same fractions analyzed by the iQ200, or were tested after an additional dilution based on reported hospital results. The iQ200 WBC concentration was determined from the lysed fraction. The RBC concentration was determined as the difference between the total cell concentration in the unlysed fraction and the WBC concentration in the lysed fraction.
Results: Linear regression analysis (iQ200 versus hemocytometer): RBC, slope=0.889 (0.852 to 0.925), intercept =−1767 (−5520 to 9054), R^2=0.962; WBC, slope=1.078 (1.043 to 1.114), intercept=10.0 (−98 to 118), R^2=0.976.
Conclusions: (1) iQ200 performance is equivalent to standard hemocytometer counts; (2) WBC identification in the presence of RBC is simplified, providing improved clinical information; and (3) iQ200 automation would reduce the workload of the laboratory while maintaining the visual benefit of microscopic analysis.

TP4.27

PROTHROMBIN TIME DERIVED FIBRINOGEN DETERMINATION ON SYSMEX CA-500 COAGULATION ANALYSER

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Aim of the study: Prothrombin time derived assays for fibrinogen are widely used because they are less expensive and come at no extra cost with prothrombin time assays. We evaluated PT derived fibrinogen determination with reference to the Clauss fibrinogen assay using a Sysmex CA-500 Coagulation Analyser. Prothrombin times were performed using the Dade Behring Thromborel S. Fibrinogen was assayed by the Clauss method using a commercial kit (Dade Behring Multifibren U).
Method: Samples were analysed from normal subjects (n=43) and hospitalised patients were divided into groups with fibrinogen concentration within reference interval (1.8–3.5 g/L; n=99) and above the reference interval (n=119), and patients receiving oral anti coagulant therapy (n=26).
Results: The results of the correlation study between PT derived fibrinogen and the Clauss assay indicated that the PT derived method is suitable for routine purposes in normal subjects ($y=0.92x+0.27$ r=0,72; p=0.71) and hospitalised patients with fibrinogen concentrations within the normal range ($y=1.06x+0.12$ r=0.70; p=0,02). However, it seems inadequate under conditions where the prothrombin time is prolonged (such as anti coagulant therapy; $y=1.28x-0{,}04$ r=0.88; p=0.001) and in the presence of high fibrinogen levels ($y=1.15x-0.06$ r=0.81; p=0.002) and is not recommended for routine clinical use.
Conclusion: The results indicate that the prothrombin time derived method is convenient for routine purposes (normal subjects) but it is not recommended for use in monitoring patients in response to their clinical status.

TP4.28

SERIAL DILUTION HELPS TO DISTINGUISH SPURIOUSLY LOW PROTEIN S ACTIVITY IN PATIENTS WITH FACTOR V LEIDEN MUTATION

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Protein S (PS) plays an important role in the regulation of blood coagulation. About 40% circulates in free form, and functions as cofactor for APC in degrading activated coagulation factors V and VIII. PS deficiencies are known to be associated with increased risk for venous thrombosis. The factor V ‘Leiden’ (FVL) mutation is present in 20% of patients with deep venous thrombosis and represents an especially high risk if combined with other risk factors. Screening for PS deficiency is part of the routine thrombophilia laboratory panel. However, technical problems often interfere with the interpretation of the PS assay.
Our aim was to formally investigate whether the presence FVL mutation interferes with the clotting-based PS activity assay used in our laboratory, and to determine if diluting the samples could help avoiding this technical problem.
Patients’ plasma with normal or decreased levels of PS were diluted in Veronal buffer, thus decreasing the ratio of patient FV in the final reaction mix, which contains PS deficient plasma with WT FV. Dilutions of 1:2 and 1:4 were evaluated. Levels of total and free PS antigen by ELISA (Asserachrom, Diagnostica Stago, France and Coaliza, Cromogenix, Italy) in stock, as well as activity (Protein S Ac, Dade Behring, Germany) in stock and diluted plasmas were determined.
Results: The dilution curves of WT and FVL samples were distinctly different in both normal and PS deficient patients. Samples with FVL displayed a much flatter curve. We conclude that using serial dilutions for FVL positive samples is a helpful tool in identifying true PS deficiency.

TP4.29

ABERRANT LIGHT SCATTER AND FLUORESCENCE PATTERNS IN DE NOVO ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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ALL is a malignant disorder that arises from a single B-or T-lymphoid cell. About 80–85% of ALLs originate from B cells or their precursors and 15–20% are of T cell origin. Between January 1, 2001 and December 31, 2004, 50 newly diagnosed ALL patients' samples have been examined in our laboratory by three color flow cytometry. Our goal was to find abnormal light scatter and fluorescence patterns (aberrant phenotype), to which the follow-up samples from patients could be compared. The samples were mainly bone marrows. Of the 50 patients 25 were children, 25 were adults, 26 were males, 24 were females. By flow cytometry, 3 cases turned out to be T-ALL, the other 47 were of B cell origin. Our examinations focused on the following leukemia associated phenotypes: lineage switch, abnormal light scatter patterns, overexpression of different antigens and cross-lineage antigen expression. We found only a single case of lineage switch. Cross-lineage antigen expression – which meant myeloid positivity in ALL – was observed in 12 cases (24%), abnormal light scatter was detectable in 35 samples (70%). Overexpression of CD10, CD34 and TdT antigens was detected in a large number of investigated cases and was studied by both qualitative and quantitative methods. Aberrant expression of CD10 was observed in 38 patients (76%), CD34 in 40 patients (80%) and TdT in 29 patients (58%). It is recommended that a multiparametric approach – utilizing both aberrant scatter and fluorescence parameters – is used in the identification of lymphoblasts especially for detection of minimal residual disease.

TP4.30

IMMUNONEPHELOMETRIC DETERMINATION OF FIBRINOGEN ON CITRATED OR HEPARIN PLASMA: COMPARISON WITH FUNCTIONAL CLAUSS METHOD

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Fibrinogen is routinely determined by functional assay on citrated plasma in Haematology departments. However, immunoassay can be performed easily with nepehelometric analysers in the clinical chemistry laboratory, allowing automation. The aim of this study was first to compare the clotting Von Clauss method (activity assay) with an immunonephelometric method (antigen assay) on the BN ProSpec™ (Dade Behring). Moreover we evaluated the possibility of collecting heparinised blood samples. The use of heparin plasma would allow minimal blood sample collection.
Initially the accuracy of immunonephelometric analytical performance was tested on heparin and citrated tubes. For comparison studies, fibrinogen activity was then determined on citrated tubes in the Haematology department, and the antigen measurement was performed on both citrated and heparin plasma from 130 consecutive patients.
As a result, the immunonephelometric method shows reliable performance (all CVs were $<5\%$). Furthermore clinical sample measurements are not affected by the method used, nor by the type of sampling validating the use of heparin plasma samples for fibrinogen antigen determination with Dade Behring reagents (correlation coefficients $r^2>0.97$).
In conclusion heparin plasma fibrinogen antigen determination gives similar results than Von Clauss fibrinogen activity evaluation, strongly suggesting that this method could be used routinely in order to evaluate inflammatory states and/or cardiovascular factors.

TP4.31

AUDIT OF REQUESTS RECEIVED BY THE SUPRAREGIONAL ASSAY SERVICE FOR ERYTHROPOIETIN

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The number of requests for serum erythropoietin (EPO) has risen exponentially over 10 years since the service was introduced and during 2004 over 2500 specimens were analysed. There are differences between hospitals regarding the format of request forms and it was thought that a customised request form for EPO would be beneficial to the service.
To assess the extent of this problem an audit was carried out in October 2001. 103 request forms from 43 hospitals were reviewed which showed that clients were using a letter (7%), a laboratory form (52%) or a computerised form (41%). The audit reviewed nine criteria for determining satisfactory service and concluded that the use of laboratory forms was problematic. The results showed that only 46% of laboratory forms gave the hospital address and 59% had a reference number. Additional criteria examined were patient demographics, inclusion of clinical details and haemoglobin level. A customised form was distributed to 12 hospitals who were regular clients (2–3 specimens per month) and using a laboratory form (11/12) or letter (1/12).
A re-audit in October 2004 reviewed 232 forms from 61 hospitals. This showed that the frequency of including a reference number was increased to 94% for clients using a laboratory form but not the hospital address (37% compliance). The King's form is being used by 10/12 hospitals and a review showed that 43 forms were received during October 2004 with $>90\%$ compliance achieved for all criteria. Data from the audit will be illustrated and measures to extend the use of the customised form will be discussed.

TP4.32

SERUM PRO-HEPCIDIN AND ITS RESPONSE TO ORAL IRON SUPPLEMETATION

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Hepcidin is a 24-amino acid peptide that is secreted from the liver. Hepcidin is involved in the regulation of iron absorption from the gut and it also causes sequestration of iron into the bone marrow macrophages. During infections the serum and urine hepcidin concentrations may be considerably elevated. Due to these biological properties hepcidin has been thought to play a role in the pathogenesis of anemia of chronic disease.

Pro-hepcidin is a 84 amino acid precursor form of hepcidin. We have evaluated the serum concentrations of pro-hepcidin using a commercial ELISA assay (DRG Instruments, Marburg, Germany). A preliminary reference range was established using a population of 39 healthy subjects. Pro-hepcidin values ranged from 122 to 1117 ng/ml in women (447 ± 206 ng/ml, mean ± S.D.) and from 97 to 782 ng/ml in men (254 ± 201 ng/ml, mean ± S.D.).

In an attempt to define the factors that determine the plasma pro-hepcidin concentrations healthy subjects were administered a 100 mg oral dose of ferrous iron ($n = 16$) and blood samples were drawn at various time-points during the following 24 h. Considerable inter-individual variation was observed in the responses of the serum pro-hepcidin. In male subjects ($n = 9$, mean ferritin 124 μg/l) no systematic changes were observed. However, in female subjects who had fairly low amounts of storage iron ($n = 7$, mean ferritin 46 μg/l), an increase in serum iron was followed by an increase in serum pro-hepcidin which, in turn, was followed by a reduction in serum iron even below the baseline level. This phenomenon suggests that the ability to absorb iron from the gut, serum iron, and serum pro-hepcidin concentrations is regulated in a coordinated manner.

TP4.33

THE EFFECT OF HORMONAL THERAPY ON COAGULATION PARAMETERS IN IN VITRO FERTILIZATION

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The hemostatic system changes in response to hormonal therapies used in preparation for in vitro fertilization (IVF).

This study aimed to analyze the impact of hormonal changes on the coagulation system in 25 women undergoing IVF.

They were treated with HMG-hCG hormones with a GnRH agonist. Effects on blood coagulation activity were determined: before hormonal stimulation, after down-regulation, at maximal estradiol level and after ovulation induction with human chorionic gonadotrophin. We evaluated the effect of estradiol levels on: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, as well as factors II, V, VII, X and XII. They were measured by coagulometric methods using a Behring Coagulation Timer.

As serum estradiol levels increased throughout next phases, significant increases were observed for fibrinogen, factors: II, V ($P < 0.05$), while we found significant decreases in the PT, aPTT and factor XII ($P < 0.05$). No significant changes were found for factors VII and X. We obtained following average values for four time points for PT: 13.6 s, 13.2 s, 12.5 s, 12.7 s; for aPTT: 36.7 s, 36.1 s, 34.2 s, 34.5 s; for fibrinogen: 2.6 g/L, 2.5 g/L, 3.3 g/L, 3.9 g/L; for factor II: 96%, 93%, 97%, 102%; for factor V: 94%, 91%, 94%, 98% and for factor XII: 99%, 95%, 93%, 95%, respectively. These findings collectively suggest that the hormone therapies used for ovarian stimulation pre IVF may alter the balance of factors in favour of coagulation.

TP4.34

SEVERE ACQUIRED FACTOR XIII DEFICIENCY CAUSED BY AN AUTOANTIBODY AGAINST THE B SUBUNIT

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Plasma factor XIII (FXIII) is a tetrameric complex (A2B2) of potentially active A subunits (FXIII-A) and carrier B subunits (FXIII-B). Acquired FXIII deficiencies caused by an autoantibody, represent rare but severe bleeding conditions.

Acquired FXIII deficiency was diagnosed in a 28-year-old female with severe intramuscular bleeding based on clinical presentation and undetectable plasma FXIII activity after excluding other haemorrhagic disorders. The aim of this study was to understand the pathomechanism of FXIII deficiency and characterize the inhibitor.

In further analysis FXIII A2B2 complex antigen level was undetectable and surprisingly, free FXIII-B level was also very low in the patient's plasma. Meanwhile, both FXIII activity and FXIII-A antigen level were normal in the patient's platelets. Using functional FXIII assay no inhibition of FXIII activity was detected in 1:1 mixture of the patient and control plasma or after supplementing the patient's plasma with purified FXIII A2B2. Furthermore, neither the activation of purified FXIII nor the activity of thrombin activated FXIII was inhibited by IgG isolated from the patient's serum, suggesting the presence of a non-neutralizing autoantibody.

In an ELISA system the patient's IgG strongly bound to purified FXIII A2B2 and to FXIII-B, while its binding to FXIII-A was negligible. These findings verified the presence of anti-FXIII autoantibody, which reacted with an epitope on FXIII-B.

In contrast to its normal half-life of 11 days, the half-life of FXIII administered as 3750 U Fibrogammin was reduced to 17 h. The results indicate that the autoantibody formed immune complexes with free and complexed FXIII-B, which were then rapidly cleared from the circulation.

TP4.35

HEMOSTASIS ABNORMALITIES IN GAUCHER DISEASE

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Gaucher disease (GD) is the most common genetic lysosomal storage disorder. The primary metabolic defect in Gaucher disease is an inherited deficiency in activity of β-D-glucosidase. The accumulation of beta-glucocerebroside in the reticuloendothelial system leading to bone marrow infiltration, skeletal lesions, hepatosplenomegaly (type I), and in rare cases to the central nervous system involvement (type II and III). Type 1 GD patients have been described with hemostasis abnormalities, contributing to

the bleeding tendency. Although usually attributed to thrombocytopenia, some patients with relatively high platelet counts and normal coagulation tests have hemorrhagic phenomena.
We performed a study of parameters of coagulation and fibrinolysis: antithrombin (AT III), plasminogen (PLG), protein C (PC), α2-antiplasmin (α2-APL), plasminogen activator inhibitor (PAI-1) in 20 patients with type 1 GD. Enzyme replacement therapy has been performed for five patients.
AT III, PLG, PC, α2-APL and PAI-1 were measured with spectrophotometric method, using Behring Coagulation Timer.
We obtained following average values (range): for AT III 113 (81–147%); for PLG 115 (86–141%); for PC 92 (53–128%); for α2-APL 88 (62–127%) and for PAI-1 2.88 (0.3–8.5 U/mL). One patient had activity of protein C less than 70%, five patients had concentration of PAI-1 above 3.5 U/mL and four patients had activity of α2-antiplasmin less than 80%.
These abnormalities in hemostatic system at some patients may be the result of consumption of coagulation factors caused by ongoing coagulation activation possibly due to mononuclear cell activation. Further studies are necessary to elucidate the mechanism behind this response.

TP4.36

NEW PARAMETERS IN EVALUATION OF PLATELET FUNCTION ON BAYER ADVIA 120

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Measurements of platelet activation during routine hematological investigations might offer advantages in the clinical evaluation and management of patients at risk for thrombosis and other diseases. Numerous anticoagulants have been used to measure platelet activation, and they have different effects on shape and structure of platelets. The Advia 120 Haematology System measures the routine platelets parameters as a platelet count (PCx109/L), mean platelet volume (MPV, fL), but offers novel parameters of platelet activation status, mean platelet component concentration (MPC, g/L), platelet component distribution width (PCDW, g/L), mean platelet mass (MPM, pg) and platelet mass distribution width (PMDW, %).
In this study, the mean values of platelet activating parameters were established and compared in different anticoagulated whole blood specimens.
Blood of 20 healthy volunteers, being on no medication (aspirin, non-steroidal and anti-inflammatory drugs), was drawn into sodium citrate and K2EDTA (Terumo) and CTAD tubes (BD). PC values were 220.7 ± 28.4, 211.8 ± 28.7, 181.6 ± 24.8 in EDTA, sodium citrate and CTAD, respectively. The MPC values were higher in EDTA (257.8 ± 21.5) than in citrate (246.7 ± 8.6) and the lowest in CTAD (232.0 ± 9.9), $p < 0.001$ among groups. PCDW as an indicator of morphological changes was the lowest in blood with EDTA (52.3 ± 3.6) and significantly higher in citrate (71.6 ± 4.18) and CTAD (77.5 ± 1.9), $p < 0.001$. There were found no differences in MPM and PMDW in each anticoagulated group. MPV was different among groups ($p < 0.001$), with the lowest values in EDTA (8.6 ± 0.7, 9.4 ± 0.7, 10.4 ± 0.5, respectively).
Since the platelet count remained consistent but their function was remarkably activated, EDTA appeared to be good for routine but not for functional investigation. CTAD and citrate as moderate activators may be useful for platelet function investigation.

TP4.37

DEVELOPMENT OF LABORATORY NETWORK SUPPORTED THE NATIONAL PREVENTION AND CONTROL PROGRAM OF THALASSEMIA IN THAILAND

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As medical laboratories have responsibility in prevention and control of thalassemia, 12 laboratories of the Department of Medical Sciences throughout Thailand have been developed to have potential to perform haemoglobin (Hb) separation and determination, α-thalassemia 1 diagnosis and create a laboratory network in their responsible area.
Results from 2001–2004 showed that sample numbers increased gradually from 4027 samples in 2001 to 12,840 samples in 2004. Blood samples that required confirmation by DNA sequence analysis were submitted to the National Institute of Health. The characterization of β-thalassemia mutations indicated the high genetic variation in the Thai population especially in the Southern part of the country. 12 β-thalassemia mutations and 13 abnormal Hb were identified. In order to maintain accuracy, reproducibility and clinically relevant laboratory testing, all laboratories performed internal quality control and participated in the national proficiency testing scheme.
After 4 years of the study; reliable, fast and effective laboratory service co-operation have been developed to support the prevention and control program of thalassemia in Thailand.

TP4.38

EFFECTS OF 8-Cl-cAMP ON HAEMATOLOGICAL PARAMETERS IN PERIPHERAL BLOOD ON WISTAR RATS

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8-Chloro-cyclic adenosine-3′,5′-monophosphate (8-Cl-cAMP) is a cyclic adenosine-3′,5′-monophosphate (cAMP) analog that specifically down regulates protein kinase A (type I) and causes growth inhibition in a wide range of cancer cells. The objective of study was analysis of effects of 8-Cl-cAMP on hematological parameters (number of erythrocytes and reticulocytes, MCV, haematocrit and hemoglobin concentration) in peripheral blood on Wistar rats.
The animals were grouped in two experimental groups and one control group. 1st experimental group composed of 16 animals (8 male; 8 female) was treated with dose of 30 mg/kg/d 8-Cl-cAMP,

2nd experimental group composed of 19 animals (10 male; 9 female) with dose of 60 mg/kg/d and control group composed of 8 male and 8 female untreated animals. 8-Cl-cAMP was applied subcutaneously, once per day. Blood samples were collected before and then at 7th, 14th, 21st and 28th day after initial application. Haematological parameters were determined by conventional techniques.
Results show cyclic changes in the number of erythrocytes in both experimental groups with a slight decrease of no more than 10% in comparison to reference values. Dose dependence was observed for number of reticulocytes also. MCV values for both experimental groups are 2–9% above reference values. The oscillatory changes in haematocrit values were registered for both experimental groups. Hemoglobin concentration showed a tendency of oscillatory decrease, exceptions were male animals treated with lower dose where the curve has a linear character.
The results suggest that effects of 8-Cl-cAMP cause dose dependent changes in values of studied haematological parameters, suggesting a role of protein kinase A in cell metabolism and inhibition of cell proliferation.

TP4.39

QUALITY CONTROL MATERIAL FOR PROFICIENCY TESTING SCHEME OF HAEMOGLOBIN SEPARATION AND DETERMINATION

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The accurate and reliable measurement of haemoglobin (Hb) separation and determination is essential for thalassemia diagnosis. The objective of this study was to validate quality control material (KKU-Hb) to be used in proficiency testing scheme of Hb separation and determination in Thailand.
Two treated EDTA blood samples prepared from a normal subject and a beta-thalassemia carrier were used. Their homogeneity and their stability in storage during April to June 2004 were validated. Six laboratories throughout the country participated in this study. Both HPLC and LPLC instruments were used for Hb separation and Hb A2 determination.
Results of the homogeneity study showed that these quality control materials had a small coefficient of variation ranging between 1.43–4.58% and 4.27–5.56% for intralaboratory and interlaboratory studies, respectively. They could be stored at -6 °C for 2 months without significant decrease in Hb A2 level (t-test, $p > 0.05$).
In conclusion, the results of homogeneity and stability support the use of these quality control materials in proficiency testing scheme of Hb separation and determination.

TP4.40

IMMUNOGLOBULIN ISOTYPE SWITCHING IN MULTIPLE MYELOMA AFTER HEMATOPOIETIC CELL TRANSPLANTATION

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The malignant plasma cell clone in patients with multiple myeloma (MM) usually produces a single abnormal monoclonal immunoglobulin with a constant isotype and light-chain restriction (paraprotein). Switching of the paraprotein isotype or transient presence of oligoclonal bands detectable by serum immunofixation electrophoresis has been reported following the high-dose chemotherapy. The presence of these additional protein bands could be related to the recovery of impaired Ig production after both autologous and allogeneic transplantations rather than to a change in the paraprotein production by the malignant plasma cell clone.
We carried out a retrospective analysis of 72 myeloma patients who underwent 138 transplantation procedures in the period from 1996 to 2002 to determine the frequency and clinical significance of the appearance of abnormal proteins bands distinct from the presenting paraprotein. 71 patients underwent the first autologous transplantation of peripheral blood cells (PKB), in one patient only a classical allogeneic transplantation from related donor was performed. In 43 (61%) patients the second autologous transplantation of PKB and in 9 (13%) patients even the third autologous transplantation were done, in 15 patients (21%) a non-myeloablative transplantation of PKB from related donor was performed as the second transplantation procedure. An apparent abnormal protein band distinct from the paraprotein present at diagnosis was found in 6 (8%) patients. 13 (18%) patients had oligoclonal bands and 14 (19%) patients had both isotype switch and oligoclonal bands after transplantation identified by immunofixation electrophoresis (SEBIA, France).
We have discussed the results of thorough analysis and a possible impact of this interesting laboratory finding on the prognosis of MM patients.

TP4.41

THROMBOPHILIA SCREENING IN SYSTEMIC LUPUS ERYTHEMATOSUS

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The aim of our study was to identify significant risk factors in a patient population with SLE, and to determine the prevalence of thromboembolic events, so relative risk could be assigned to these factors.
Data from 53 patients with SLE were studied. A detailed thromboembolic history was taken. Sera, plasma and DNA samples from all patients were analyzed with an extensive screening for thrombosis risk factors including antiphospholipid-antibodies, APC resistance, factor V Leiden and prothrombin mutations, protein C, protein S and antithrombin activity, factor VIII and VWF levels.
In our study population, the presence of anti-phospholipid antibodies, factor V Leiden mutation, and elevated factor VIII level were found to confer a relative risk (RR) for thrombosis of 3.4, 3.4 and 2.7, respectively. The combination of all three

carries an extreme high risk for the development of thromboembolism.
SLE patients are routinely tested for anti-phospholipid antibodies. Our results raise the possibility that screening of anti-phospholipid antibody positive patients for other thrombotic risks should be evaluated. We plan to validate our results on a larger patient population, however, firm recommendation can only be based on randomized prospective data.

TP4.42

A NEW EXTERNAL QUALITY ASSESSMENT (EQA) SCHEME FOR INTRINSIC FACTOR ANTIBODY (IFAb) ASSAYS: A KEY DIAGNOSTIC CRITERION FOR PERNICIOUS ANAEMIA

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The diagnosis of Pernicious Anaemia (PA) is based on the haematological features of megaloblastic anaemia in conjunction with a low serum cobalamin (B12). 50% of PA patients have a detectable antibody to IFAb causing B12 malabsorption.
The 'gold standard' test for diagnosis is the Schilling test. The non-availability of the reagents in 2002–2003 has heightened the importance of IFAb as a diagnostic tool.
The Scheme collected samples from patients having B12 < 180 ng/l and measured IFAb by 2 ELISA methods and an in house binding method.
Selected samples were also assayed for Gastric Parietal Cell antibody (GPCAb).
68 samples had B12 < 180 ng/l. 7 out of 68 gave positive IFAb by the ELISA methods but only 3 patients had positive results by both ELISA methods. 4 of the 7 positive patients' sera were assayed by the in house method and equivocal or negative results were recorded. 5 of the 7 positive patients' sera were tested for GPCAb but only 3 gave positive results.
Requests from laboratories lead UK NEQAS to introduce an IFAb EQA scheme. The anomalies, illustrated by the 3 methods tested, suggested that the designated response should be assigned by clinical and laboratory criteria.
The first scheme survey of 105 laboratories resulted in 103 in agreement with the designated response. Inter method differences are already apparent in further surveys.
Some of the sensitivity and specificity anomalies may be due to interference by elevated B12 levels or contamination of kit reagents with gastric parietal cells. The Scheme plans to investigate both of these phenomena.

TP4.43

ACOUSTIC MEASUREMENT OF ERYTHROCYTE SEDIMENTATION RATE AND OTHER PARAMETERS OF BLOOD WITHOUT REAGENTS

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An acoustic method for tests without reagents of erythrocyte sedimentation rate, hemoglobin concentration, red cell count, red cell indexes, white cell count and platelet count has been developed by Russian producers of medical devices. The acoustic method is based on the measurement of the speed and absorption rate of ultrasonic waves in blood. The method has been realized in the device called BIOM/Russia/. The received signal from the ultrasonic waves passed through the device cell with patient blood is adapted by means of an original mathematical model. The results are calculated on a PC and presented on printouts.
The aim of the present work is to evaluate the feasibility of the acoustic method in clinical medicine for simultaneous measuring hemoglobin concentration, red cell count, red cell indexes, white cell count, platelet count and erythrocyte sedimentation rate in blood without reagents for screening.
More than 600 samples of patients' blood have been tested. Acoustic determination of erythrocyte sedimentation rate has been compared with the results obtained by Westergren method, cell count—with the tests performed by Sysmex SE-9000/Japan/ Hematology Analyzer. At present time the high correlation coefficients have been obtained for the hemoglobin concentration ($R = 0.99$, $p < 0.05$), the RBC (red blood cells)-$R = 0.92$, $p < 0.05$ and for the HCT (Hematocrit) $R = 0.96$, $p < 0.05$. The device is able to measure the erythrocyte sedimentation rate for only 5–10 min of exposure.
It has been suggested that the method can be used in clinical medicine for examination of anemia and elevation of the erythrocyte sedimentation rate. The methodical aspects of such measurements (WBC, PLT) are being developed.

TP4.44

CHARACTERIZATION AND STUDY OF DIFFERENTIAL PHENOTYPE EXPRESSION OF A FAMILIAL CASE OF PRIMARY ANTIPHOSPHOLIPID ANTIBODY SYNDROME IN ADULTS TO THE 2ND GENERATION ASSOCIATED WITH HYPERHOMOCYSTEINEMIA AND CONGENITAL DEFICIENCY OF PROTEIN S

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Design: History: A nuclear family with familial pattern of Primary Antiphospholipid Antibody Syndrome associated with hyperhomocysteinemia and Congenital Deficiency of Protein S with two generations affected (mother and all sons). First is a 35-year-old-woman, second is a 30-year-old-woman and third a 26-year-old-woman.
Methods: Family tree (history), familial pattern, clinical characteristics and laboratory data included: antiphospholipid antibodies (enzyme immunoassay), MTHFR genotype (PCR and hybridization with specific probes for 677 position), prothrombin genotype (20210 position), Factor V LEIDEN genotype, homocysteine levels and special coagulation studies were obtained.
Results: Clinical characteristics: 6 recurrent abortions, (1st) gestation of 30 weeks (2nd) placental detachment and fetal death,

postnatal ocular thrombosis and intolerance at acenocumarol (3rd). Laboratory data: antiphospholipid antibodies: positive. MTHFR genotype: heterozygote for mutation in 677 position C-T. Prothrombin genotype and Factor V LEIDEN genotype: no mutation. Coagulation studies: INR normal, APTT prolonged. Special coagulation: deficiency of Protein S and hyperhomocysteinemia in all cases, thrombocytopenia (3rd), hyperuricemia (4th). Conclusion: (1) We describe for the first time an association between a familial case of Primary Antiphospholipid Antibody Syndrome in adults to the 2nd generation, hyperhomocysteinemia and Congenital Deficiency of Protein S. (2) We observe a family in which differential phenotype of Primary Antiphospholipid Antibody Syndrome expressed was: recurrent abortion (1st) placental detachment, intrauterine death, postnatal ocular thrombosis and intolerance at acenocumarol (3rd). (3) Prophylactic treatment with standard heparin during pregnancy was given. (4) Primary Antiphospholipid Antibody Syndrome, hyperhomocysteinemia and congenital deficiency in coagulation inhibitors is associated with an increased risk of vascular disease and is a cause of hereditary thrombotic disease.

TP4.45

EVALUATION OF CELL DYN 1800 HEMATOLOGY ANALYSER

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Hematology instruments are expected to give accurate and reproducible results for a wide variety of clinical conditions and to provide these results without unnecessary delay.
Cell Dyn 1800 (Abbott Diagnostics, USA) is a new automated hematology analyser that provides an 18-parameter blood count. The throughput is 60 tests per hour. The instrument is smaller than its predecessor and offers a variety of enhanced features, including increased patient data storage, availability of a bar code reader, and a cyanide-free reagent system. The analyser was evaluated by comparing results obtained from the Cell Dyn 1600 CS and the Cell Dyn 3500 SL.
The performance of the Cell Dyn 1800 was evaluated in a district hospital laboratory using the guidelines proposed by the Finnish Labquality. The Cell Dyn 1800 results showed excellent correlation ($r > 0.976$) compared to Cell Dyn 3500 SL with the following parameters: white blood cells, red blood cells, haemoglobin, mean corpuscular volume and platelets. The Cell Dyn 1800 lymphocyte and granulocyte cell count also showed excellent correlation with the results of the Cell Dyn 1600 CS ($r > 0.984$) and Cell Dyn 3500 SL ($r > 0.977$). In contrast mid-cell fraction showed poorer correlation with the corresponding results in the Cell Dyn 1600. The results of the Cell Dyn 1800 demonstrated minimal carryover. Precision was generally acceptable for all CBC parameters (CV < 6%).
The results clearly show the excellent performance of the Cell Dyn 1800 and that the instrument can be recommended for routine use in small laboratories and as a back-up or emergency analyser in medium sized laboratories.

TP4.46

THE EFFECT OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON GRANULOCYTE ENZYMES ACTIVITY IN CHILDREN WITH NEUTROPENIA AFTER CHEMOTHERAPY IN THE COURSE OF CANCER

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Granulocyte-Colony Stimulating Factor (G-CSF) is a cytokine that stimulates proliferation and maturation of granulocyte precursor cells. The results of the investigations in vitro and in vivo conducted on animal models found that this cytokine influences the functions of mature granulocytes which can be confirmed by the activity of the granulocyte enzymes participating in phagocytosis. An attempt has been conducted to evaluate the influence of Granulocyte-Colony Stimulating Factor on the activity of monitoring granulocyte enzymes in vivo in humans.
The investigation was conducted on a group of 26 children with cancer after injection of G-CSF for treatment of chemotherapy-induced neutropenia. The control group included 29 healthy children. The blood was taken with heparin before G-CSF application (time 0) and on 3rd and 6th days of treatment. The activity of granulocyte enzymes, myeloperoxidase, acid and alkaline phosphatase and esterase participating in the process of phagocytosis was evaluated.
It was found that Granulocyte-Colony Stimulating Factor affects the activity of granulocyte enzymes by normalising lower values of myeloperoxidase, acid phosphate and increasing the correct values of alkaline phosphate activity. The activity of enzymes increases during the days of treatment. On the basis of obtained results we can conclude that Granulocyte-Colony Stimulating Factor apart from granulopoiesis has also the ability to activate the non-specific cellular immunity in vivo in humans.

TP4.47

MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) - THE EXPERIENCE OF DEPARTMENT OF LABORATORY DIAGNOSTICS, REGIONAL HOSPITAL IN POZNAN, POLAND

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Aim: To assess the frequency of occurrence of MGUS among all monoclonal gammopathies (MG) recognized by “protein criteria” and to evaluate the malignant transformation of MGUS during 36 months of follow-up.
Material and methods: All newly diagnosed MG were included (years 1993–2003). Among cases of MGUS the group monitored for 3 years by measuring a concentration of serum monoclonal protein and Bence–Jones proteinuria level was isolated. Proteins measurements were performed by means of electrophoresis, immunoelectrophoresis, immunofixation; the concentration of

serum immunoglobulins and Bence–Jones proteinuria were measured by means of kinetic turbidimetry and nephelometry.
Results: Out of 1690 cases of MG, 431 (25%) fulfilled "protein criteria" of MGUS: 212 female (49%) and 218 male (51%). We found 319 (74%), 59 (14%) and 53 (12%) cases with IgG, IgA and IgM monoclonal protein, respectively. Among 431 patients with MGUS 112 (26%) were monitored by proteins investigations. The group of 59 patients monitored for 36 months was isolated (32 female and 27 male). During follow-up 11 (18%) cases developed malignancy, 25% among female and 11% among male. In IgG, IgA and IgM MGUS cases, malignancy developed 15%, 27%, 25%, respectively. The interval from the time of recognition of the M-protein to the malignant transformation ranged from 1 to 27 months (median 15 months).
Conclusions: High frequency of MGUS indicates the importance of patient monitoring. Comparing to literature data, more cases of MGUS developed malignant transformation. Higher percentage of progression MGUS to malignant MG observed in this study may come from a lower number of cases being monitored. Women and patients with IgA and IgM M-proteins showed an increased risk of progression.

TP4.48

RETICULOCYTE INDICES IN IRON DEFICIENCY ANEMIA

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The enumeration of peripheral blood reticulocytes is a crucial step in defining the primary cause of anemia.
We examined a control group of 55 nonanaemic adults and 79 patients with iron deficiency anaemia (IDA). Iron deficiency was confirmed by ferritin and soluble transferrin concentration. Reticulocyte indices were obtained on the Advia 120 hematology analyzer (Bayer Diagnostics).
The values of reticulocyte hemoglobin content (Ret-He), corrected reticulocyte percent (CRI) and mean cellular volume of reticulocyte (MCVr) were 31.2 ± 1.5 pg, $1.3 \pm 0.5\%$ and 104.5 ± 4.7 fL for the control and 23.3 ± 3.0 pg, $0.8 \pm 0.5\%$ and 92.9 ± 8.4 fL for IDA patients, respectively. By performing a *T*-test significant differences in means of these parameters were found between control and anemic group ($p < 0.01$). Reticulocyte maturity is quantified based on increased RNA content, and presented as immature reticulocyte fraction (IRF). IRF is defined as the fraction of reticulocyte with highest content of RNA (IMR-H) and with the highest and medium content of RNA (IRF-H+M). In IDA patients IRM-H ($7.2 \pm 5.2\%$) and IRM-H+M ($19.0 \pm 7.8\%$) were significantly higher than IRM-H ($2.5 \pm 1.9\%$) and IRM-H+M (12.3 ± 4.8) in control ($p < 0.01$).
These results lead to the conclusion that although the reticulocyte number is decreased in IDA, maturation is disturbed, and increased numbers of immature reticulocytes are released from the bone morrow. Their haemoglobin content and cellular volume are lower which will result in the production of hypochromic, microcytic erythrocytes. Complete insight into morphology of the reticulocytes can predict the course of iron deficiency anemia.

TP4.49

FUNCTIONAL EXAMINATIONS AND THEIR PREDICTIVE VALUE FOR IDENTIFICATION OF ANTIPHOSPHOLIPID SYNDROME

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Antiphospholipid antibodies (APA) represent a heterogenous group of antibodies, which form complex compounds either with negatively charged phospholipids or their complexes with macromolecular substances. Functional tests are used for the laboratory identification of APA, because of their ability to affect coagulation tests dependent on phospholipids. If the tests are abnormal, a dilution study is carried out (patients' plasma and standard plasma mixture).
We used the following functional tests: the Thromboplastin test (Prothrombin time – PT), APTT, APTT-LA sensitive to LA type antibodies and the Russel's Viper Venom time – RVVT. Commercially available diagnostic tests were used (PT: Grifols, Spain; APTT, APTT-LA and dRVVT: Stago, France). All coagulation studies were performed on the Compact analyser (STAGO, France) which detects the end of the coagulation process.
We compared the functional tests with a test which directly determines the hexagonal phospholipids levels in plasma (STACLOT LA, STAGO, France). We sought to find out a predictive value for the presence of LA type antibodies for individual functional tests and for some of their ratios. We compared two groups, one with positive tests (25 respondents) and one with negative results (173 respondents). The correlation relationships dependencies between STACLOT LA and individual tests were not confirmed. A statistically significant increase ($p < 0{,}01$) was proved in all functional tests. An interesting result was observed in comparing the APTT-LA/APTT ratio (r) for identification of hexagonal phospholipids – STACLOT LA (positive test: $r = 1.39 \pm 0.29$, negative test: $r = 1.02 \pm 0.12$, $p < 0.01$). This finding shows that the ratio r could be a predictive factor in selection of tests used for the examination of antibodies against Lupus Anticoagulant.

TP4.50

SERUM FREE LIGHT CHAIN (FLC) RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS)

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Aim: To assess the impact of the serum FLC ratio on progression of MGUS to malignancy.

Methods: 1148 MGUS patients diagnosed between 1960 and 1994 had cryopreserved serum samples that had been collected within 30 days of MGUS diagnosis. At a median follow-up of 15 years, malignant progression occurred in 87 patients (7.6%). The initial diagnostic sera were assayed for FLC (FREELITE™, The Binding Site Limited, Birmingham, U.K.) on a Dade Behring BNII.
Results: An abnormal FLC ratio (kappa/lambda ratio <0.26 or >1.65) was detected in 379 patients (33%). The risk of progression in patients with an abnormal FLC ratio was significantly higher compared to patients with a normal ratio (hazard ratio, 3.5; 95% CI, 2.3–5.5; $p<0.001$) and in multivariate analysis was independent of the size and type of the serum monoclonal (M) protein. Patients with an abnormal serum FLC ratio, IgA or IgM MGUS, and a high serum M protein level (≥ 1.5 gm/dL) had a risk of progression at 20 years of 58% (high-risk MGUS) ($n=53$ patients), versus 37% with any two of these risk factors (high-intermediate-risk) ($n=226$), 21% with one risk factor (low-intermediate risk) ($n=420$), and 5% when none of the risk factors were present (low-risk) ($n=449$).
Conclusions: The FLC ratio is a significant predictor of malignant progression in MGUS, and is independent of the size and type of the serum M protein. The low-risk subset accounts for almost 40% of all patients with MGUS and will impact management.

TP4.51
DIFFERENCES IN HEMATOLOGY PARAMETERS IN DIABETIC PATIENTS OF SARAJEVO GENERAL HOSPITAL
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Studies related to differences in hematology parameters in a diabetic population are very rare. Few studies relating good control of diabetes mellitus and thrombocyte number, or control of function of blood cells have been reported so far. A rise in glycemic control in patients with type 2 diabetes causes a reduction in thrombogenicity. Coagulability is highly increased in these patients. It is believed that good glycemic control can help in correction of this hyper-coagulation status.
In this study, on the basis of erythrocyte, leukocyte and thrombocyte counts, as well as other parameters, the differences between two types of diabetes were analysed in relation to a control population. Blood samples of 60 patients with the diagnosis of diabetes mellitus (30 types 1 and 30 of type 2) and 60 patients within reference values of glucose were used in this study. Glucose was analyzed with the GOD method, while hematology parameters were analyzed with the laser counter Cell Dyn 3200. Liquid cytometric analysis was the basis for detection.
Our studies show an increase in hematocrit, leukocyte, lymphocyte and monocyte numbers in diabetic patients, while MCV, MCH and neutrophil numbers are decreased. Patients with type 2 diabetes show an increase in erythrocytes, HCT, leukocytes, lymphocytes and glucose with respect to controls. Hematocrit and thrombocytes are significantly increased while neutrophils and monocytes are decreased in patients with type 2 diabetes type in relation to patients with type 1 diabetes. In male samples studied, an increase in glucose concentration affects the values for MCV, MCH and eosonophils; in women it reflects an increase in basophil number and hemoglobin concentration.

TP5: LIPIDS AND LIPOPROTEINS

TP5.01 SELECTED FOR POSTER CLINIC
MICROSATELLITE MARKERS AND HAPLOTYPE ANALYSIS OF THE R145C MUTATION IN THE APO E GENE IN DYSBETALIPOPROTEINEMIA
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Apolipoprotein E (ApoE) is a ligand for the receptor-mediated uptake of remnant particles of triglyceride-rich lipoproteins; a reduction in its binding affinity or its absence leads to dysbetalipoproteinemia. This phenotype is commonly due to homozygosity for the ApoE2 isoform (Cys112, Cys158) but other mutations are known including a substitution at position 145 (R145C). This particular mutation is common in black South Africans. The aim of this study was to distinguish between a single or multiple origin for the mutation by setting up non-denaturing polyacrylamide gel electrophoresis (PAGE) for the analysis microsatellite lengths.
Patients ($n=47$) and families ($n=3$) from the lipid clinics at the university as well as controls ($n=30$) were studied. Microsatellite markers were amplified by PCR and sized by non-denaturing PAGE. Haplotypes were constructed by combination of two microsatellites and one intragenic single nucleotide polymorphism (SNP).
Microsatellite genotyping by non-denaturing PAGE was shown to be a viable alternative to the more popular denaturing PAGE, as genotypes and haplotypes at the locus could be constructed and were shown to segregate in the three kindreds chosen for analysis. There was no major common haplotype for the R145C allele in black patients. A shared haplotype was found for the mutant allele in two kindreds studied.
These data are unable to distinguish between a single or multiple origin for the R145C mutation at the ApoE locus in black South Africans. Microsatellite markers are known to mutate rapidly so it is possible that a mutation happening very early in the history of black South Africans would carry a diversity of microsatellite lengths in present descendents.

TP5.02 SELECTED FOR POSTER CLINIC
THE MOLECULAR AND CELLULAR DEFECT UNDERLYING AUTOSOMAL RECESSIVE HYPERCHOLESTEROLEMIA (ARH) IN SOUTH AFRICA
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Defective low density lipoprotein (LDL) clearance manifests as familial hypercholesterolemia (FH) with the clinical phenotype of premature atherosclerosis. Inheritance is autosomal dominant with the defect lying with the LDL receptor. A second protein in the pathway, the LDLr adaptor protein (ARH), facilitates interaction of the LDLr with the protein matrix required for receptor internalization and is implicated in a rare autosomal recessive form of hypercholesterolemia. The aim of this project was to confirm the diagnosis of ARH in a black South African.

Blood was taken from the subject for DNA isolation with a skin biopsy being taken for tissue culture. Defective LDLr function was excluded, as cultured skin fibroblasts from the subject were able to take up and degrade labelled LDL normally.

The coding sequence of the ARH gene was determined by sequencing cDNA derived by RT-PCR of fibroblast mRNA.

A single G insertion was found in a run of Gs in exon 1. This insertion changes the reading frame and introduces a premature stop codon at amino acid 33, severely truncating the ARH protein. Two single nucleotide polymorphisms (SNPs) were also found. One lies in exon 6 (C→ T) and substitutes proline for serine while the second occurs in exon 7 (G→ A) and is silent. This latter SNP is novel, as it does not appear in any SNP database.

The establishment of a PCR based assay for the mutation now allows for carrier detection in the family and for an assessment of the carrier frequency in the broader population.

TP5.03 SELECTED FOR POSTER CLINIC

NOVEL RISK FACTORS FOR ACUTE MI IN INDIANS

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Objective: Indians have a high prevalence of coronary heart disease. Traditional risk factors do not account for this very high prevalence. We looked at some of the novel risk factors in patients with acute myocardial infarction.

Study design: The design of the study was case control. 50 consecutive cases of acute MI were recruited. Two controls were recruited for each case and were age matched. Subjects with any history of heart disease in the past were not recruited as controls. Fasting blood samples were taken for measurement of Apolipoprotein A, Apolipoprotein B, lipoprotein (a), homocysteine, oxidized LDL, total antioxidant and hsCRP. Serum was stored at −70 °C before analysis. Analysis of apolipoprotein A, Apo B and lipoprotein (a) was by immunoturbidimetric method. Homocysteine, oxidized LDL and CRP were done by ELISA. Total antioxidant measurement was by ABTS method.

Results: The mean age of the cases and controls were not different. In cases with MI a significantly higher mean hsCRP (6.88 ± 3.92 mg/L) was evident as compared to controls (2.49 ± 3.18 mg/L). An increase in apo B (122.43 ± 40.02 mg/dl vs. 102.96 ± 30.00 mg/dl) and a decrease in mean Apo A1 (112.77 ± 38.58 mg/dl vs. 141.40 ± 33.80) levels were observed in cases as compared to controls. We did not however find a difference in Lp (a) levels between the two groups. The circulating oxidized LDL was significantly elevated in cases as compared to controls (35.35 ± 19.91 vs. 29.80 ± 13.45 U/L) and this was accompanied by a lower total antioxidant capacity in cases with MI. No difference in homocysteine levels was evident although the mean homocysteine was higher than the upper cut off for normal in both cases and controls suggesting an underlying folate/B12 deficiency.

Conclusion: The study suggests that novel risk factors like Apo B, hsCRP and oxidized LDL could be important in addition to the traditional risk factors in the development of CAD in Indians.

TP5.04 SELECTED FOR POSTER CLINIC

GENE/ENVIRONMENT INTERACTIONS AND LIPID METABOLISM

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Fibrates increase high density lipoprotein cholesterol (HDL-c) by acting as ligands to PPARα, which regulates the transcription of genes encoding proteins involved in triglyceride rich lipoprotein and lipoprotein lipase metabolism. Whilst treatment with fibrate drugs is usually associated with an increase in HDL-c, there is much variability in response and genetic factors have been implicated in this. Rarely a paradoxical fall in HDL-c following the initiation of fibrate therapy is observed. Our hypothesis was that a paradoxical fall in HDL-c might reflect genetic abnormalities in the PPARα gene or the Peroxismal Proliferator Response Element (PPRE) in the promoter regions of target genes (apo A-I, apo A-II, LPL, SR-B1 or ABC-A1). The PPARα gene and the promoter regions of the target genes were sequenced in 15 fibrate-treated patients with a paradoxical HDL-c response and 15 controls. In addition mutations in the promoter region of apoAV, a highly responsive PPARα target gene, were investigated.

This study revealed a total of 30 known and novel polymorphisms in the genes sequenced. There was a greater frequency of heterozygotes for SNPs in intron 2a (37602 C>A) and (37673 C>G) of the PPARα gene in the paradoxical group ($p = 0.04$). In addition, there was a significant difference ($p = 0.03$) in genotypes for ApoA-II -265T>C and also a significant difference in allele frequencies at this site, with the T allele more prevalent ($p < 0.01$) in cases.

In conclusion, we identified a number of polymorphisms, three of which may be associated with the paradoxical fibrate response. However we did not identify any mutations in the PPRE of the genes sequenced which would explain the paradoxical response.

TP5.05 SELECTED FOR POSTER CLINIC

BEXAROTENE – ANOTHER CAUSE OF IDIOSYNCRATIC HYPOALPHALIPOPROTEINAEMIA (LOW HDL SYNDROME)

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Low plasma HDL cholesterol (<1.0mmol/L) is an independent risk factor for CHD. Fibrates, acting via the nuclear receptor heterodimer PPARα-RXRα, are known to both lower plasma triglyceride and

increase HDL Cholesterol levels. However, occasional patients treated with fibrates have paradoxical effects on these parameters, including a marked reduction in HDL cholesterol levels. This phenomenon has also been observed in association with Thiazolidinediones, which are PPARα agonists. A number of novel compounds, which are known RXR selective agonists, are actively being investigated as potential therapeutic agents for diverse conditions including T2DM, skin and malignant diseases. Bexarotene is one such drug, which has recently been introduced for the treatment of advanced cutaneous T-cell lymphoma. This agent is known to cause reversible central hypothyroidism in treated patients and it also adversely affects the lipid profile, causing a mixed hyperlipidaemia. While there may also be an accompanying reduction in HDL cholesterol levels, we report a case of a 58 yr old male with stage IV cutaneous lymphoma in whom the introduction of Bexarotene produced a profound reduction in plasma HDL cholesterol (<0.2 mmol/L), in the presence of moderate mixed hyperlipidaemia (Triglycerides <8.0mmol/L). Moreover, there was an associated pronounced reduction in plasma Apo AI, but not in Apo AII. This patient also manifested central hypothyroidism, which was actively treated.

This represents the first documented case of hypoalphalipoproteinaemia in association with the therapeutic use of a RXRα selective agonist. Furthermore, this observation implicates the RXRα nuclear receptor in the pathogenesis of the "low HDL syndrome". Finally, this paradoxical action of Bexarotene may have implications for the development of specific RXRα agonists as therapeutic agents for the treatment of T2DM.

TP5.06 SELECTED FOR POSTER CLINIC

LDL SIZE AND OXIDIZED LDL MAY BE BETTER MARKERS OF CAD THAN CONVENTIONAL LIPIDS ALONE

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Hyperapobetalipoproteinemia (HBL) characterized by increased LDL-apoB with normal LDL-c is one of the most common lipoprotein disorder associated with cardiovascular diseases. Lower levels of LDL-c/LDL-apoB ratio (≤1.2) reflect the preponderance of small-dense LDL-particles which are more prone to oxidation.

Oxidation of low-density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis. It has been utilized as a marker of oxidative modification of proteins in atherosclerosis. How oxidized lipids affect the initiation and progression of atherosclerotic lesion is still largely unknown. The presence of small-dense LDL in combination with elevated apoB is reported to increase Coronary Artery Disease (CAD) risk to 6.2 fold.

25% of our study subjects fall into HBL category. Keeping this population in mind the present study was undertaken to evaluate the role of apo-B, ox-LDL and LDL particle-size along with conventional lipids in CAD.

Angiographically verified CAD patients ($N=72$) and healthy controls ($N=85$) constituted the study group. The conventional lipids were estimated with fully enzymatic kits on an autoanalyzer. Ox-LDL and apo-B levels were estimated by immunoassay. LDL particle-size was estimated by calculating the ratio of LDL-c/LDL-apoB levels.

Among the conventional lipids only HDL-c ($P=0.013$) discriminated patients from controls, whereas ox-LDL ($P=0.000$), plasma apoB ($P=0.05$), LDL-apoB ($P=0.001$) and LDL-c/LDL-apoB ($P=0.032$) ratio levels were significantly higher in patients than in controls. Multivariate Analysis of the studied parameters revealed that ox-LDL {RR=8.00 (3.3–19.5, 95.0%CI)} and LDL-size {R$R=7.00$ (3.1–15.8, 95.0%CI)} are better risk markers for CAD.

This preliminary fact clearly indicates that small LDL-size and elevated levels of ox-LDL may be better indicators of CAD risk than conventional lipids alone.

TP5.07

LIPID AND ANTIOXIDANT SYSTEM IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Objective: To evaluate the antioxidant status of children with familial hypercholesterolemia (FH) before and after antioxidant treatment.
Antioxidant use: β-carotene 12–30 mg/d (0.05 mg/kg/d).
Methods:

*Obs.period: 3 months
*Age:3–15 years
*Serum β-carotene, vitamin E and A levels of 41 children with FH and 11 controls were measured by HPLC.
*Plasma thiobarbituric acid reactive system (TBARS) was measured by fluorimetric method. Serum selenium and zinc concentration was determined by AAS.
*Serum total and LDL cholesterol and triglyceride levels were measured by enzymatic methods.
*Statistical analysis by Mann–Whitney U Test; Statistical significance $p<0.05$.

Result:

1. Before antioxidant and antilipemic treatment β-carotene and TBARS level were higher, selenium level was lower in FH children as compared to control.
2. FH children treated by antilipemic diet alone had lower zinc and higher selenium and TBARS levels than FH children on the same diet supplied by β-carotene.

Conclusion:

1. Lipid peroxidation was increased in FH children without a lack of vitamin E, selenium and β-carotene.

2. The antilipemic diet together with β-carotene may decrease the lipid peroxidation of FH children.

TP5.08

LIPOPROTEIN (A), APOLIPOPROTEIN A-I AND B SERUM LEVELS IN SCHOOL-AGED CHILDREN FROM SERBIA

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In the last few years it has been proved that risk factors for atherosclerosis are present in children and adolescents. The aim of this study was to evaluate levels of atherosclerosis risk factors such as lipoprotein (a) (Lp(a)), apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) in school-aged children.
Lp(a), Apo A-I and Apo B levels were estimated by use of immunoturbidimetric methods. The study group consisted of 624 children divided according to the age into three groups: 7 (91 girls, 99 boys), 10 (111 girls, 101 boys) and 13 yrs (106 girls, 116 boys). Means Apo A-I and Apo B were not different between sexes. The level of Apo A-I in the oldest group (1.45 ± 0.40 g/L) was significantly higher versus 7 yrs (1.34 ± 0.38 g/L) and 10 yrs group (1.37 ± 0.29 g/L). In group aged 10 yrs was the higher mean of Apo B (0.91 ± 0.31 g/L) than in other two groups: 0.80 ± 0.32 g/L in 7 yrs and 0.80 ± 0.31 g/L in 13 yrs group. Significantly higher level of Lp(a) was found in 13 yrs old girls (0.33 ± 0.35 g/L) than in same age boys (0.18 ± 0.18 g/L). In groups of youngest children there was not significant difference in Lp(a) means between sexes. The mean in 7 yrs group was 0.27 ± 0.17 g/L and in 10 yrs 0.22 ± 0.21 g/L. Correlation analysis showed that the levels of Lp(a), Apo A-I and Apo B were not depended on BMI.
In view of these special attention should be paid to primary prevention.

TP5.09

IDENTIFICATION OF APO(a) PHENOTYPES IN MACEDONIAN POPULATION

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Elevated Lipoprotein (a) [Lp (a)] concentrations are positively correlated with risk of premature development of coronary heart disease (CHD).
The aims of our study were electrophoretic separation and identification of Apo(a) isoforms, estimation of allele frequencies of Apo(a) phenotypes and determination of Lp(a) concentration.
180 healthy blood donors were included in the study, aged 18–60. Separation and visualization of Apo(a) isoforms were done by 3–15% polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS–PAGE) followed by immunoblotting. Lp (a) concentrations were assessed immunonephelometrically.
Estimation of allele frequencies (LpO, LpB, LpS1, LpS3, LpS4, Lp>S4) was done by Maximum likelihood method using EM algorithm.
The frequency distribution for plasma Lp (a) concentrations in healthy Macedonians was highly skewed, with a mean of 15.542 mg/dL and a median of 9.62 mg/dL. As expected, there was a statistically significant inverse correlation between Apo (a) isoform size and Lp (a) plasma level ($r = -0.3477$, $p < 0.001$).
Carriers of null phenotype accounted only for 5%, single and double band phenotypes were observed in 59.44% and 35.56% of subjects, respectively. The most frequently were HMW isoforms: S4 (49.14%) and S3 (27.59%) from single banded isoforms and S4S3 (43.75%) and >S4S4 (34.38%) from double-banded isoforms.
The present study provides the first evidence on the allele frequencies of Apo (a) phenotypes in healthy Macedonian subjects. The calculated Apo(a) allele frequencies were: LpO 0.242; LpB 0.022; LpS1 0.028; LpS3 0.201; LpS4 0.397; Lp>S4 0.110. Comparison of the observed phenotype frequencies versus expected ones, showed that there is no statistically significant difference (chi-square of 17.21, $df = 15$, $p < 0.3067$).
The present results show that Macedonian population is in Hardy-Weinberg equilibrium.

TP5.10

DOES TERIPARATIDE ADVERSELY AFFECT THE "LIPID PROFILE"?

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CHD is also a major contributor to both morbidity and mortality in the elderly. Moreover, it has been clearly demonstrated that an adverse plasma lipid profile is a key risk factor in the aetiopathogenesis of CHD. Osteoporosis is also well established as a cause of morbidity in this cohort. Teriparatide (recombinant PTH 1–34) is an increasingly used novel and efficacious treatment for postmenopausal osteoporosis. However, it has been reported by the manufacturers (Lilly) that hypercholesterolaemia is a common adverse event (<10%) associated with the use of Teriparatide. In addition, hypercholesterolaemia is noted as a side-effect of Teriparatide in the National Osteoporosis Society patient information sheet.
In this pilot study we examined the effect of Teriparatide on our routine lipid profile, ApoAI and ApoB, in a cohort of 20 subjects (53–90 years) undergoing treatment. Fasting samples were obtained immediately prior to initiating Teriparatide and 3 months after commencing treatment.
In relation to plasma cholesterol, calculated LDL cholesterol, triglycerides, non-HDL cholesterol and ApoB, there was no significant difference observed in mean levels before and 3 months post commencement of Teriparatide. However, mean HDL cholesterol significantly improved with treatment (1.65 mmol/L (pre) vs. 1.78 (post) mmol/L, $p < 0.02$), although mean ApoAI levels were not significantly different between the groups (1.49 vs. 1.46, $p =$ ns).
Our data indicate that hypercholesterolaemia is not a widespread problem associated with the use of this Teriparatide. Furthermore, there may be some beneficial effect on HDL cholesterol levels, which, rather intriguingly, opens up the possibility of a mechanistic

link between lipid and calcium metabolic pathways. A larger study is required to further elucidate this observation.

TP5.11

POTENTIAL BIOMARKERS FOR ATHEROTHROMBOSIS IN PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Thrombotic tendency of the antiphospholipid syndrome shares several pathways with atherothrombosis. The aim of this study was to analyze the influence of: antibodies (anticardiolipin, anti-β2glycoprotein I, anti-oxLDL antibodies, which were detected by ELISA, and lupus anticoagulant, which was detected by coagulation tests), lipids (total serum cholesterol, LDL, HDL and triglycerides) and potential biomarkers for atherothrombosis (apolipoproteins AI, B, Lp(a), high-sensitivity CRP, which were detected by immuno-nephelometry, and homocysteine, which was detected by HPLC method) on clinical characteristics of 33 patients with primary antiphospholipid syndrome (mean age 41 ± 14), and to compare analyzed parameters in patients and in 28 healthy subjects (mean age 37 ± 12). Patients with a history of arterial thromboses had higher concentrations of triglycerides ($p < 0.001$), cholesterol ($p < 0.05$), and lower concentrations of HDL ($p < 0.05$). Higher concentrations of LDL ($p < 0.05$), cholesterol ($p < 0.05$), and apolipoprotein B ($p < 0.05$) were present in patients with a history of cerebrovascular insults. Patients with myocardial infarctions had higher concentrations of homocysteine ($p < 0.05$). In patients who were positive for anti-oxLDL antibodies, venous thromboses were associated with lower levels of apolipoprotein AI ($p < 0.05$); while thromboses of small blood vessels were associated with the presence of anti-β2glycoprotein I antibodies of the IgM isotype ($p < 0.05$). According to our results, which have to be confirmed in a larger study, testing the above parameters is justified in patients with primary antiphospholipid syndrome with a view to introduction of additional therapy for minimizing the probability for recurrent thrombotic episodes.

TP5.12

DEVELOPMENT OF A RAPID LIPOPROTEIN FRACTIONATION PROCEDURE TO INVESTIGATE CHANGES OF LIPOPROTEIN COMPOSITION AND DENSITY DISTRIBUTION

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Ultra-centrifugation is widely used in lipoprotein analysis as an analytical and preparative tool. Traditional floatation and salt gradient methods are time consuming and require large sample volumes. Iodixanol is a novel non-ionic iso-osmotic compound capable of forming self-generating, reproducible and stable gradients. Using a VTI90 vertical rotor in a Beckman Optima XL-100K ultracentrifuge we have developed an iodixanol gradient (9%(2 ml)/20%(1.5 ml) iodixanol with 1.4ml overlay of HEPES buffer) offering rapid (75 min), reproducible lipoprotein separation using small volumes of patient plasma (0.75 ml).

20, 250 μl fractions were collected across the density gradient formed and the density distribution of the plasma lipoproteins was determined by analysis of their cholesterol, triglyceride, apo B-100 and apo A-1 content.

Expressing the cholesterol recovered in each fraction as a percentage of the total recovered in all fractions proved to be most effective in demonstrating differences in the lipoprotein profiles and subclass density distribution between individuals and the populations studied.

Lipoprotein profiles were examined in fasting plasma from non-diabetics with low total:HDL cholesterol ratios (controls) ($n = 16$); non-diabetics with a raised total:HDL cholesterol ($n = 8$) and type II diabetics ($n = 25$). As expected, significant differences were observed in LDL and HDL density distribution between controls and the two test populations with a shift to greater density in these fractions associated with increasing plasma triglyceride and the total:HDL cholesterol ratio. Diabetics with markedly elevated triglycerides (>7.0mmol/L) showed a significant difference in HDL density distribution, but not in LDL density distribution, when compared to diabetics with lower triglycerides.

TP5.13

EFFECTS OF PRAVASTATIN TREATMENT ON sICAM-1 AND sVCAM-1 LEVELS IN HYPERCHOLESTEROLEMIC SUBJECTS

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Objective: Lipid-lowering therapy with 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase inhibitors reduces the incidence of atherosclerosis-related cardiovascular events. Adhesion molecules (AM), regulating interaction between vascular and circulating cells, may play a central role in the pathogenesis of atherosclerosis and related complication. Whereas an association between hypercholesterolemia and AM expression has been suggested, it is unclear whether lowering cholesterol decreases AM expression and release. In the present report we examined the effect of the pravastatin on serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1) in 30 individuals with hypercholesterolemia who presented with no other risk factors or evidence of atherosclerosis.

Methods: We determined serum levels of sICAM-1 and sVCAM-1 using an enzyme immunoassay. Fasting lipid profile, sICAM-1 and sVCAM-1 were measured before and after 2 months post-pravastatin treatment (20 mg/day).

Results: Compare to baseline pravastatin administration significantly reduced low-density lipoprotein levels (7.0012 ± 2.0481

mmol/l versus 5.0327 ± 1.8279; 28% reduction; $P < 0.001$), LDL/HDL ratio (5.73 ± 1.7 versus 3.64 ± 1.08; 36% reduction; $P < 0.001$). Furthermore, treatment with pravastatin significantly diminishes sVCAM-1 (690.41 ± 137.63 ng/ml versus 305.5 ± 87.64 ng/ml; $P < 0.001$) and sICAM-1 (516 ± 132.59 ng/ml versus 227.83 ± 57.46 ng/ml; $P < 0.001$).

Conclusions: Lipid-regulation effects of pravastatin completed with pleotropic effects which demonstrated in reduce levels of soluble adhesion molecules. The reduction in levels of AM may be indicative of less activated state of the subendothelium which possibly may contribute to modulation of the progression of atherosclerosis.

TP5.14

HOMOCYSTEINE AND CARDIOVASCULAR RISK IN HEMODIALYSIS PATIENTS

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Hyperhomocysteinemia is associated with the precocious development of cardiovascular disease and is one of the risk factors that increase the mortality in the end stage renal disease. The aim of the study was to evaluate the total homocysteine (tHcy) levels with respect to the haemodialysis stage.

The plasma tHcy was measured by fluorescence polarization immunoassay, on Abbott AXSYM analyser (cut-off 10.2 mmol/L) in the following haemodialysis patients: study group I, $n = 42$ (29 male and 13 female), average 59.4 ± 16.3 years with mean haemodialytic age 92.4 ± 18,6 months, study group II, $n = 56$ (38 male and 18 female) average 53.7 ± 16.4 years and mean haemodialysis age 46.3 ± 13.8 months. One half of each group of patients was treated with folic acid for six months.

There was no significant difference with respect to age, sex and BMI in all haemodialysis patients. Statistically higher plasma tHcy levels were found among patients with and without folic acid supplement ($p < 0.002$) 38.3 ± 14.2 mmol/L vs. 29.4 ± 12.3 mmol/L. Significantly lower tHcy levels were found in patients on folic acid supplement however they where higher in patients with longer haemodialysis age, and the values were 27.5 ± 14.3 mmol/L vs. 18.6 ± 10.2 mmol/L ($p < 0.001$) in the other stage group.

Folic acid supplement was effective in lowering tHcy, but in patients with mean haemodialysis age of 92.4 ± 18.6 months, tHcy levels remained statistically higher than the reference range, and were associated with increase of cardiovascular risk.

TP5.15

LIPOPROTEINS AND CORONARY HEART DISEASE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction: Patients with Rheumatoid arthritis (RA) are under substantially increased risk of death from cardiovascular diseases compared with age-matched individuals from the general population.

The aim of this study was to determine the incidence of cardiovascular disease in patients with RA by determination the lipoprotein (a), Lp (a), and other lipoprotein parameters; total cholesterol (TC), low density cholesterol (LDL-c), High-density cholesterol (HDL-c), triglycerides (TG), apolipoprotein A-I and B and CRP status on base line and after 12 weeks of therapeutic management. The study group includes: 28 Resohin, 32 Methotrexate and 16 Dekartin-cortico medication patients, both sexes from 32–68 of age, and 25 healthy controls were assessed.

Results: There were a significant increases in serum Lp (a) ($p < 0.001$); TG ($p < 0.01$); apo B ($p < 0.01$); TC and LDL-c ($p = 0.01$) than controls patients. In 62% of RA patients and 8% of controls had Lp (a) concentrations greater than 30 mg/dl. Also, increased values of CRP ($p < 0.01$) and decreased HDL-c level were found in patients with RA. In addition, there was a significant positive correlation between CRP status and Lp (a) in the study group ($r = 0.698$, $p < 0.001$), but not in controls. Conclusion: There was no significant difference of lipoprotein parameters before and after anti-inflammatory medication. Increased Lp (a) and the other lipoprotein levels suggest an additional risk factor for cardiovascular progression and a potential adjuvant therapy.

TP5.16

INTERACTIVE EFFECT OF HEPATITIS C VIRUS-SEROPOSITIVITY AND LIPOPROTEIN(A) ON OCCURRENCE OF CORONARY ARTERY DISEASE

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Hepatitis C virus (HCV) role in the pathogenesis of atherosclerosis is still discussed. Moreover, possible underlying mechanisms that might explain the association between HCV and atherosclerosis also remain unclear.

Our aim was to investigate whether HCV-seropositivity (HCV(+)) and its association with elevated lipoprotein(a) (Lp(a)(+), >30 mg/dl) levels may have a role in coronary artery disease (CAD).

Blood samples were tested for HCV(+) (Assam, Abbott, USA) and elevated Lp(a) levels (Beckman, USA) in 581 patients undergoing coronary angiography (158 without coronary artery atherosclerotic lesions, No-CAD, and 423 with CAD).

HCV(+) percentage resulted 2.5% and 6.8% in No-CAD respect to CAD ($p < 0.05$), increasing with the number of affected vessels (4%, 8.1% and 9.3% for one-, two- and three vessel-disease, $p < 0.05$). Univariate analysis showed that, in addition to other traditional atherogenic risk factor (age, gender, smoking habit, hypertension, diabetes and dyslipidemia), HCV(+) and Lp(a)(+) were associated with CAD (odds ratio, OR: 2.8, $p < 0.05$, and 2.2 $p < 0.001$).

After adjustment for the other risk factors, HCV(+) and Lp(a)(+) still represent independent predictor for CAD (OR: 3.4, $p < 0.05$ and 2, $p < 0.01$).

Increasing CAD prevalence was seen depending on HCV(+) and Lp(a)(+) (68% for both negative, 81% either positive, 100% both positive, $\chi^2 = 14.7$, $p < 0.001$).

These results provided evidence that HCV-seropositivity may represent an independent risk factors for CAD. Moreover, elevated Lp(a) levels were found to affect the risk of CAD prevalence in HCV-seropositive patients. Thus, the research of specific risk factors profile may assist in the identification of subgroups of patients in which infection might play a prominent role in the atherosclerotic process, and that might eventually benefit of an appropriate pharmacological therapy.

TP5.17

SERUM APOB AND LDL-C IN SERA WITH DIFFERENT TG RANGES

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The aim of the study was to evaluate relation between serum apoB and LDL-C in groups of patients divided according to cholesterol (121–200 group I, 201–250 group II, >250 mg/dl group III) and TG levels (6 groups with increasing serum TG from <100 to 400mg/dl. Materials and methods. In 583 patients in whom lipid profile was requested TC, TG, HDL-C and LDL-Cd by direct method, apoB were measured by standard methods from Roche. LDL-Cc according to Friedewald formula and apoB/LDL-Cd and apoB/LDL-Cc were calculated. Results. Serum apoB increased along with increasing TC and LDL-C. Significant increase in apoB/LDL-C was observed in Group I-III with the highest values in group I and II with TG above 251 mg/dl in which ratio apoB/LDL-Cc was higher than apoB/LDL-Cd (1.2 versus 0.97 in group I with TG>300 and 0.99 versus 1.11 in group with TG>250) In high cholesterol and high TG groups neither LDL-C direct and apoB/LDL-Cd nor LDL-Cc and apoB/LDL-Cc showed significant differences. Conclusions. The relation between apoB, TC, LDL-C was proved. In normal and border line TC ranges with TG above 250 mg/dl apoB/LDL-C calculated from Friedewald formula reaching the values above 1.0 may be the indicator of the false LOW results of LDL-C. In those cases direct LDL-C measurement should be applied. Serum apoB and apoB/LDL-Cc may be helpful in finding the TG concentration which may be accepted for LDL-C evaluation especially in patients with low and border range of total cholesterol.

TP5.18

EFFECTS OF ROSUVASTATIN ON SERUM HOMOCYSTEINE, VITAMIN B12 AND FOLIC ACID LEVELS IN PATIENTS WITH PRIMARY HYPERLIPIDEMIA

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Hyperhomocysteinemia is regarded as an independent risk factor for cardiovascular disease. Lipid-lowering agents (mainly fibrates) can modify homocysteine (Hcy) levels. There is limited information about the effects of the recently introduced rosuvastatin on Hcy.

To investigate the effects of rosuvastatin on total Hcy, vitamin B12 and folic acid in patients with primary hyperlipidemia.

A total of 28 patients (10 men and 18 women) with primary hyperlipidemia (total cholesterol >240mg/dl, and triglycerides <350 mg/dL) were assigned to rosuvastatin 10 mg/day. Serum lipid and metabolic parameters, including Hcy, vitamin B12 and folic acid, were measured at baseline and after 8 and 16 weeks on rosuvastatin treatment. Moreover, creatinine clearance (CrCl) was calculated by means of Cockroft-Gault formula.

Statin treatment produced significant reductions in total, LDL-cholesterol, apolipoprotein B and triglyceride concentrations, whereas HDL-cholesterol, apolipoprotein A-I and lipoprotein(a) levels did not significantly change from baseline. There were no significant variations in renal function parameters (serum creatinine and CrCl) during the period of treatment. No significant differences were observed in serum concentrations of Hcy (9.3 ± 3.5 vs. 9.5 ± 3.5 vs. 9.1 ± 3.7 μmol/l, p=NS), vitamin B12 (324.3 ± 91.0 vs. 327.8 ± 102.3 vs. 328.3 ± 98.2 pg/ml, p=NS) and folic acid levels (12.2 ± 2.8 vs. 12.7 ± 2.7 vs. 12.2 ± 2.5 ng/ml, p=NS) were noted in the course of rosuvastatin treatment.

Besides its lipid lowering efficacy, rosuvastatin has a neutral effect on serum homocysteine levels, which may be in favor of its "cardioprotective" properties.

TP5.19

APOPROTEIN H POLYMORPHISM IN DYSLIPIDEMIC SUBJECTS SHOWING DIFFERENT LDL PARTICLE SIZE DISTRIBUTION

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It has been shown that small, dense LDL is a potent atherogenic lipoprotein that can be used to improve risk prediction and evaluate response to lipid therapy. The mechanism(s) responsible for their production remain unclear. The possible role of apolipoprotein H (also named β2-glycoprotein I) in production of those small, dense LDLs has been investigated in last few years. Previous studies have shown that apolipoprotein H is a regular structural component of the major classes of lipoproteins, has a high affinity for triglyceride-rich particles, stimulates the removal of plasma triglycerides, and activates lipoprotein lipase. In view of these findings, the aim of our study was to evaluate the possibility that the alterations in LDL particle size are influenced by structural polymorphism of apoH molecule. We determined the frequency distributions of the three apoH alleles in 386 subjects with various dyslipidemias that were later classified also according to the predominant LDL pattern: pattern A with predominant large LDL particles (n=127), intermediate pattern (n=111) and pattern B with small LDL particles (n=148). A group of 138 healthy blood donors with normal lipid profile and normal distribution of LDL particle size (pattern A) was selected as a control group for the evaluation of apoH protein polymorphism in our population. LDL size phenotyping was performed by non-denaturing horizontal gradient polyacrylamide gel electrophoresis developed in our laboratory. ApoH phenotypes were determined by isoelectric focusing followed by immunoblot-

ting. Our results showed no significant differences between the distribution of apoH phenotypes in control subjects and dyslipidemic subjects with different LDL particle size distribution.

TP5.20

EFFECT OF HALF MARATHON ON SERUM LIPID LEVELS IN UNTRAINED MEN AND WOMEN

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The mobilization of glycerol and free fatty acid (FFA) from adipose tissues though lipolysis is important for the regulation of energy balance during exercise. The change in lipid metabolism during exercise has been examined in trained males or females. It is known that blood creatine kinase, lactate, and FFA levels in trained and untrained persons are increased by exercise loading. It is generally accepted that serum HDL-cholesterol (HDL-TC) level is higher in females than in males. It is, however, unclear whether exercise loading such as marathon causes different changes in blood lipid levels in untrained males and females. Therefore, we examined the effect of half marathon on serum lipid levels in untrained males and females. Blood was collected from 20 persons (10 males; 10 females) just before and 30 min after half marathon. Serum total cholesterol, HDL-TC, phospholipid, triglyceride, and FFA were measured by commercial clinical test kits. There were no differences in serum total cholesterol, HDL-TC, phospholipid, and triglyceride concentrations between pre- and post-half marathon in males and females. Serum FFA concentrations in males and females increased just after half marathon but the rate of increase in the concentration was higher in females than in males. Although the increase serum FFA concentration decreased 30 min after half marathon in males and females, the rate of decrease in the concentration was higher in females than in males. These results indicate that there is a clear difference in the change in serum FFA concentration after half marathon between untrained males and females.

TP6: MISCELLANEOUS

TP6.01

HIGH-ALTITUDE PULMONARY EDEMA SUSCEPTIBILITY IS ASSOCIATED WITH VARIANTS OF GENES OF VASCULAR HOMEOSTASIS

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Travelers experience various levels of discomfort at high-altitude due to hypoxia. High-altitude pulmonary edema (HAPE) is a severe form of altitude illness developing in sojourners upon rapid ascent to altitudes >2500 m. Since, HAPE involves enhanced vascular reactivity to exercise and hypoxia, the genes of vascular homeostasis are expected to play a key role in its pathogenesis.

We investigated the association of potential polymorphisms in the genes of blood pressure regulation with HAPE. The Angiotensin-converting enzyme (ACE) I/D, Angiotensinogen (AGT) M235T, T174M, Angiotensin II receptor type-I (AGTR1) A1166C and G2228A, Aldosterone synthase (CYP11B2) -344T/C, intron-2 conversion, K173R and C5160A, endothelial Nitric oxide synthase (NOS3) G894T and 4B/4A VNTR and Endothelin-1 (EDN1) (CT)n-(CA)n repeat, T2288G and -/A polymorphisms were investigated. The subjects comprised of 59 HAPE patients (HAPE-p), who developed the disease at 3400 m, 64 HAPE resistant controls (HAPE-r), who had been to altitudes of >3400 m 2–3 times, and 136 Himalayan natives (HLs).

The ACE II genotype was significantly greater in the HLs and HAPE-r than HAPE-p ($P<0.05$). The CYP11B2 5160A allele, NOS3 894T and 4A alleles, EDN1 (CT)n–(CA)n repeat 27 associated significantly with HAPE ($P=0.02$, 0.03, 0.04, 0.04, respectively). Wild-type alleles 894G and 4B of NOS3 were significantly higher in the HLs than HAPE-p ($P<0.0001$). Significantly higher ACE ($P<0.001$), aldosterone ($P=0.05$) and Endothelin-1 levels ($P<0.001$) and lower NO levels ($P<0.0001$) correlated with HAPE. The results indicate that certain variants of the genes of vascular homeostasis are associated with HAPE susceptibility. The findings may have applications in the health-care of travelers, mountaineers, tourist industry workers and participants in endurance sports.

TP6.02

LIPOSOMES AS POSSIBLE CARRIERS FOR ANTI-TUMORAL AGENTS

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Liposomes are a biocompatible and biodegradable drug system that affords significant benefits over conventional delivery methods. They are good candidates for local targeting of therapeutic agents to the site of interest while reducing systemic toxicity and minimizing the side effects.

The aim of our study was to establish the liposomal formulation suitable to entrap two potential anti-tumoral compounds, lactoferrin (Lf), an iron-binding glycoprotein, and NB-DNJ a protein glycosylation inhibitor. The effect of liposome-entrapped agents on the growth and morphology of murine melanoma B16-F1 cell line was also investigated.

We found that Lf (500 μg/ml) entrapped in negatively charged pH-sensitive liposomes reduced by 70% the number of living cells and induced morphological changes typical to an apoptotic process as revealed by Tunel method.

No significant effect on the growth and morphology of the cells was observed in the presence of 50 μM of entrapped NB-DNJ but the enzymatic activity of tyrosinase, the key enzyme of the melanin biosynthesis pathway, was reduced about sevenfold. These results are very interesting since the protein glycosylation in tumors such as skin cancer is believed to be different from that in the normal tissues.

TP6.03

THE EFFECT OF QUERCETIN ON TOPOTECAN CYTOTOXICITY IN MCF-7 AND MDA-MB 231 HUMAN BREAST CANCER CELLS

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Topotecan is a Camptothecin derivative which shows a large spectrum of antitumor activity. Topotecan exerts its cytotoxic effect on tumor cells mainly by inhibition of topoisomerase I activity resulting in double-strand DNA breaks. In our study, we investigated the combined cytotoxic action of Topotecan and Quercetin in MCF-7 and MDA-MB 231 human breast cancer cells. In order to examine the possible relation between the cytotoxic activity of topotecan and oxidative stress, we measured reactive oxygen species (ROS) and nitrite levels in both human breast cell lines. MCF-7 and MDA-MB 231 cells were exposed to Topotecan, Quercetin or a combination of both agents for 24 h at 37 °C. The viability of the cells was measured using the colorimetric MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. We determined ROS and nitrite levels as indicators of oxidative stress in both cell lines with and without Topotecan and/or Quercetin incubations using fluorometric dichlorofluorescein diacetate (DCFH-DA) and diaminonaphtalene (DAN) assay. The IC50 concentration of topotecan was 100 ng/mL in MCF-7 cell line and 160 ng/mL in MDA-MB231 cell line. Treatment with Quercetin enhanced cytotoxicity of Topotecan 1.4-fold in MCF-7 and 1.3-fold in MDA-MB-231 cell line. A significant increment in ROS and nitrite levels was found in MCF-7 and MDA-MB-231 cells following Topotecan incubation. Our results suggest that Topotecan has cytotoxic activity against both the breast cancer cell lines in vitro. Combination with Quercetin increases the efficacy of Topotecan in the treatment of breast cancer. Our results indicate that increased oxidative stress may play a role in the cytotoxic action of Topotecan.

TP6.04

IN VITRO TESTING OF A NOVEL TYPE OF ANTITHROMBOTIC COMPOUNDS WITH THROMBIN INHIBITORY AND FIBRINOGEN RECEPTOR ANTAGONISTIC ACTIVITY IN THE SAME MOLECULAR ENTITY

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Cardiovascular diseases are the leading cause of mortality and morbidity in the Western world. Therefore, a search for novel antithrombotics as an alternative to the existing treatments of these diseases remains a challenging pharmacological goal.
A novel type of potentially antithrombotic compounds with inherent thrombin inhibitory and fibrinogen receptor antagonistic activity has been developed. The pharmacophores D-Phe-Pro-Arg and Arg-Gly-Asp were merged into one molecule which will bind to the thrombin active site and also mimic the Arg-Gly-Asp sequence of fibrinogen and so bind to the platelet fibrinogen receptor. These new compounds were tested in vitro for their inhibitory activity against thrombin and factor Xa in purified enzyme–inhibitor–chromogenic substrate systems, by blood coagulation assays (prothrombin time, activated partial thromboplastin time and thrombin time) and for their antiplatelet activity by inhibition of platelet aggregation induced by ADP and collagen.
Thrombin inhibitory potencies of these compounds were in low micromolar range (Ki values between 3.7 and 53.8 μM) and were comparable to those for factor Xa (Ki values in a range 8.8–54.6 μM). Several compounds showed also inhibition of platelet aggregation with the best compound exerting inhibitory potency of IC50 (ADP)=63 μM and IC50 (collagen)=85 μM (IC50=concentration of compound showing 50% inhibition of platelet aggregation).
In conclusion, a combination of anticoagulant and antiplatelet activity in the same molecular entity presents as a new promising approach in a search for novel antithrombotic agents.

TP6.05

A DECADE OF CARDIAC MARKER AUDIT IN THE NORTHERN REGION

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The Northern Region Clinical Biochemistry Audit Group began the audit process for cardiac markers in October 1994. In the initial survey 12/14 labs offered CK (often as part of a cardiac enzyme panel), but only 7/14 offered CK-MB, with 9/14 offering no out of hours cardiac marker service. 4/14 had agreed guidelines. Draft standards were developed with re-audit in 1998. Overall compliance against the standard categories (scored for repertoire, guidelines, sample timing, turnaround time and IQC/EQA policy) had improved from 67% to 77% over time.
Updated standards were used for re-audits in 2003 and 2004. The scores against similar categories were 93% and 97% respectively. All labs are providing a Troponin assay (2/14 Trop I), 5/14 on an open access 24 h basis, and with most having procedures to allow analysis at any time after discussion. One lab also supports POCT Troponin. 13/14 labs have protocols jointly agreed with clinicians. Areas highlighted for improvement by the latest audit include (i) an identified evidence base for protocols (5/14 at present) (ii) greater consistency in the levels used for interpretation. Concern about the functional sensitivities of Troponin assays compared with the reliance being placed on low results by local cardiologists has lead several labs to re-assessment. 9/14 labs can now quote an internally derived functional sensitivity typically around 0.03 μg/L for Troponin T.
This work has demonstrated the benefit of repeated re-audit against developing standards, reflecting changing clinical expectations, to ensure equivalence of service provision across a region. It also highlights the dynamic nature of our discipline with changes in analytes, turnaround expectations of users, increasing use of shared guidelines and the need to ensure evidence -based practice.

TP6.06

IS LOW SERUM IRON ALWAYS A MARKER OF IRON DEFICIENCY?

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Introduction: Serum iron (sFe) is involved in a variety of vital processes in the body, from cellular oxidative mechanisms to oxygen transport and delivery to body cells. The remaining body iron is present in flavoproteins, ion sulphur proteins, and as storage in iron-ferritin and transport iron transferrin.
The aim of the study was to investigate the relationship between acute inflammation and serum iron status.
Materials and methods: The study included 57 healthy individuals (group H) and 63 patients with acute inflammation (group AI). C-reactive protein (CRP) >8 mg/L was considered to indicate inflammation. sFe, CRP and serum ferritin (sF) were determined by Olympus Diagnostica GmbH (Ireland) assays (sFe by photometric color test, CRP and sF by immunoturbidimetric test); Iron saturation (Fesat) was calculated as (Fe/TIBC) × 100 using Olympus 2700 on-analyzer software.
Results: Median values of all analytes showed significant between-group differences (Mann–Whitney, $p<0.001$). In group AI, correlations were observed between sFe and Fesat ($r=0.904$), Fe and sF ($r=-0.311$), Fesat and sF ($r=-0.592$), and CRP and sF ($r=-0.311$) (Spearman, $p<0.001$). In group AI, clinical accuracy expressed by ROC analysis showed AUC and cut-off values for sFe (0.994; ≤8.4 μmol/L), CRP (1.0; >8.7 mg/L), sF (0.828; >158.8 μg/L) and Fesat (0.953; ≤23.5%) with specificity of 100%, 100%, 86.0% and 91.2%, and sensitivity of 100%, 93.7%, 68.3% and 90.5%, respectively.
Conclusion: An inverse relation of CRP and sFe concentrations, and of CRP and Fesat values in acute inflammation not accompanied by anaemia (hemoglobin within the reference range) appears to suggest that a low serum iron level need not always reflect iron deficiency in the body.

TP6.07

HAEM OXYGENASE-1 INDUCTION IN CULTURED RENAL CELLS (A704) IN RESPONSE TO OXIDATIVE STRESS DUE TO 6-HYDROXY DOPAMINE

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Urinary dopamine (DA) and its oxidation product 6-hydroxy dopamine (6-OHDA) have been reported to be increased in type 2 DM patients with proteinuria. Oxidative stress is one of the chief potential pathways for cellular damage. The cellular response to oxidative damage can be seen in the haem oxygenase (HO) enzyme system and can be reliably determined by messenger ribonucleic acid (mRNA) expression of inducible HO-1 isoenzyme.
Cultured renal cell lines (A704) were exposed to varying concentrations of 6-OHDA and DA and messenger RNA (mRNA) expression of the oxidative stress marker gene, haem oxygenase 1 (HO1), was measured by reverse-transcriptase polymerase reaction (RT-PCR).
Exposure of A704 renal cells to 6-OHDA showed dose- and time-dependant increases in HO1 mRNA induction with concentrations of 200 nM ($p<0.005$), and 400 nM ($p<0.00005$) for 2 h exposure ($p<0.002$) and 24 h exposure ($p<0.0002$) as compared to controls. DA exposure showed initial significant HO-1 mRNA inductions with 200 nmol/L ($p<0.002$) and 2 h exposure ($p<0.004$), but no further increase in mRNA induction was seen on increasing the concentration to 400 nmol/L or exposing the cells for 24 h.
The renal cell culture studies show unequivocal evidence of oxidative stress due to 6-OHDA and DA exposure, resulting in a stress response as evidenced by HO-1 mRNA induction. The stress response appears to be proportional to both concentration and time of exposure. Both dopamine and 6-hydroxydopamine are secreted in much larger amounts in nephropathy than in health and may have a potential role in causing or exacerbating the nephropathy.

TP6.08

PRODUCTION OF SELF-ASSEMBLING LANTHANIDE LABELED PROTEIN NANOPARTICLES IN BACTERIA

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Labeled synthetic nanoparticles are widely used in bioaffinity assays. Production of these particles requires many manufacturing and quality assurance steps. We demonstrate here production of apoferritin-based self-assembling nanoparticles in bacterial cells. On the outer surface these particles display biotin-carboxyl-carrier protein (BCCP) which biotinylates in *E. coli*. Particles contain europium in their core and biotin on the surface. These nanoparticles were evaluated as a labelled component in an assay for biotinylated monoclonal antibody (mAb).
Apoferritin is spherical, hollow protein with outer and inner diameters 12 nm and 8 nm, respectively. Apoferritin consists of 24 subunits N-terminal ends of which are located on the outer surface of the protein cage. BCCP is fused to N-terminal end of each subunit. Biotinylated BCCP-apoferritin subunits are produced into inclusion bodies in simple bacterial fermentation. Inclusion bodies are purified using a generic protocol. Subunits are denatured in pH 2 and Eu (III+) ions are added. At pH 8.5 BCCP-apoferritin subunits self-assemble to produce Eu (III+)-containing, biotinylated nanoparticles. Particles are then mixed with streptavidin in stochiometric molar ratio which results in particles covered with streptavidin. Testing of particles was done by first attaching biotinylated mouse monoclonal antibodies onto a solid surface using anti-mouse IgG-coated microtiter wells. Bound biotinylated mAbs were then detected with streptavidin-coated apoferritin-nanoparticle containing Eu (III+) as time-resolved fluorescent reporter.
We were able to produce functional protein nanoparticles suitable for detecting biotinylated molecules. The use of this kind of functional nanoparticles provides advantages over synthetic nanoparticles because their production is simple, fast and inexpensive.

TP6.09

AN AUDIT INVESTIGATING THE DIFFERENTIAL DIAGNOSIS OF HYPONATRAEMIA IN A DISTRICT GENERAL HOSPITAL

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Hyponatraemia is the most common electrolyte disorder in medicine. However, when patients present with this condition a differential diagnosis is seldom carried out and the cause of the hyponatraemia is rarely identified.

The aim of this audit was to establish how well clinicians at Bassetlaw District General Hospital investigate patients presenting with hyponatraemia. For this study hyponatraemia was classified as mild (sodium 120–129 mmol/L), moderate (115–119 mmol/L) and severe (<115 mmol/L).

Eighty patients were assessed in this study. Sixty-nine patients (86%) were aged 60 years or over. Sixty-one patients (76%) presented with mild hyponatraemia, seven patients (9%) with moderate and the remainder (15%) with severe hyponatraemia. All patients in the latter group were over 60 years (median age 79 years). In the majority of patients clinical signs or symptoms did not correlate with the severity of the hyponatraemia.

Only thirteen patients (16%) had a urine sodium concentration measured during the hyponatraemic episode. Fewer estimations were taken for plasma and urine osmolality. The cause of the hyponatraemia was frequently attributed to either diabetes and/or the use of diuretics. In 59% of cases a specific cause was not identified.

Although the majority of patients (88%) recovered, nine patients died. These patients presented with mild or moderate hyponatraemia. Only five of the nine patients had the cause of the hyponatraemia diagnosed.

As a consequence of this audit, local guidelines are being developed to ensure a differential diagnosis for hyponatraemia is conducted.

TP6.10

INDICATORS OF INCREASED FREE RADICAL ACTIVITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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In addition to inflammation, two further processes are important in the pathogenesis of chronic obstructive pulmonary diseases (COPD): imbalance between proteinases and antiproteinases and oxidative stress. The lungs are prone to oxidative damage as they are exposed to an oxygen-rich environment and are continually exposed to environmental toxins that stimulate phagocytic cells in the lung to generate reactive oxygen species and free radicals (FR) which cause lipid peroxidation. We have therefore measured plasma malondialdehyde-like material, i.e. thiobarbituric acid-reactive substances (TBARS) in patients with COPD. This may give some indication of lipid peroxidation, an injurious process to membranes mediated by FR. We have also measured plasma thiol concentration (PSH). Thiols are thought to act as FR scavengers and reduction in plasma thiol levels may reflect oxidative stress.

The study included 35 pts with COPD (20 men and 15 women, mean age 63 years) and the control group of 20 healthy persons, nonsmokers, mean age 54 years. TBARS were measured by spectrophotometric method with thiobarbituric acid and PSH was measured by the method of Ellman. The 2 groups were compared statistically using the Mann–Whitney U test. Our results showed that COPD pts had significantly higher TBARS level ($p<0.001$) and lower thiol concentration ($p<0.01$) in comparison with the control group. The finding of two independent abnormalities believed to reflect oxidative stress suggests that free radical activity may be increased in COPD.

TP6.11

PRODUCTION OF BIOLOGICAL NANOPARTICLES FOR DIAGNOSTIC APPLICATIONS

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Synthetic nanoparticles are typically employed in bioaffinity assays to improve assay sensitivity. Usually they are labelled latex particles with binding molecules on the surface. However these particles are laborious and slow to synthesize and there is always variation between batches. We have developed a new method to produce biological nanoparticles featuring many advantages compared to traditional synthetic particles. These self-assembling particles are based on bacteriophage structure and produced in bacterial cells enabling simple, fast and inexpensive procedure.

In this work bacteriophage T4, a bacterial virus of *Escherichia coli*, was the basis of the particle structure. BCCP (biotin carboxyl carrier protein) was used as a binding molecule and was expressed on the bacteriophage capsid surface by fusion with HOC (highly antigenic outer capsid protein) of T4. BCCP was biotinylated in vivo during bacterial cultivation. Enzyme label was targeted inside the phage capsid by fusing firefly luciferase with CTS (capsid targeting sequence). After purification the particles were incubated with streptavidin. The particles were detectable by measuring luminescence and gave a linear dose–response curve in heterogeneous assay using biotinylated antibody as analyte.

The high affinity of biotin-streptavidin interaction was used here as a model for the novel nanoparticles. In the future other binding molecules for clinical analytes and other labels as well can be applied for more specific nanoparticle applications.

TP6.12

THREE NOVEL MISSENSE MUTATIONS AND THEIR FUNCTIONAL CONSEQUENCES IN TWO SEVERE INHERITED FACTOR X DEFICIENCIES

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Inherited factor X (FX) deficiency is a rare bleeding disorder with heterogeneous molecular genetic background. We have investigated two children with severe intracranial bleeding and bruising. The patients had prolonged PT and APTT and plasma FX activity and antigen levels were below the limit of detection in both cases. Direct fluorescent sequencing of FX gene revealed three novel point mutations. Patient 1 was homozygous for a Gly204Arg amino acid exchange in a highly conserved domain of FX protein. Patient 2 was compound heterozygote for Thr233Met and Trp308Leu amino acid exchanges. Site-directed mutagenesis was performed and wild type and all mutant FX molecules were expressed in HEK293 cells. Only wild type FX was secreted into the cell media. Immunofluorescent staining and pulse-chase analysis demonstrated that all mutants were synthesized, but the cells were unable to secrete them, they were retained intracellularly and suffered degradation. Double immunofluorescent staining was used to detect the intracellular localization of mutant FX. Immunodetection of FX was combined with staining for protein disulfide isomerase (ER marker) or Golgi 58K protein. Accumulation of Arg204 FX did not follow the localization of ER while there was a good co-localization with the Golgi suggesting that Arg204 FX is mainly trapped there. Molecular modeling and energy minimization calculation demonstrated that in the Arg 204 mutant the formation of a small disulfide-bridged loop (between Cys201 and Cys206) requires more energy. In addition, Arg204 distorts part of the light chain and decreases its interaction with the heavy chain.

TP6.13

EVALUATION OF QUANTIA IMMUNOTURBIDIMETRIC REAGENTS FOR ABBOTT CLINICAL CHEMISTRY SYSTEMS

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Objective: In an increasing number of countries there is a trend to consolidate the different laboratories of a hospital in a single large setting. However the smaller lab does still play an important role. Instrument manufacturers, in accordance, tend to increase their machine capabilities producing larger and/or modular instruments to fulfil these requirements. We extensively evaluated the new Quantia line of immunoturbidimetric assays for the Abbott AEROSET® and ARCHITECT c8000® system.
Method: The following 12 analytes were validated for the use on the Abbott Clinical Chemistry Analyzers: A-1-AGP, A-1-Antitrypsin, ASO, B2microglobulin, D-Dimer, Digitoxin, Ferritin, Gentamicin, IgE, Lp(a), Myoglobin and RF. NCCLS and IFCC guidelines have been followed to assess precision at 3 different levels, detection limit, quantification limit, linearity, prozone effect, interferences, on-board and calibration stability. Method comparison studies were performed using as reference assays the Quantex reagents line (Biokit SA) on the ILab 600 Clinical Chemistry System (Instrumentation Laboratory).
Results: Total CVs, for Level 2 and 3, were all below 4%. For Level 1, the lowest, were below 5% except for D-Dimer (6.26%) and IgE (14.5%). The reason for this latter figure being that the level assayed (45 IU/mL) was very close to the quantification limit. Bilirubin up to 19.4 mg/dL (free) and 20.5 mg/dL (conjugated), haemoglobin up to 460 mg/dL, triglycerides up to 1327 mg/dL and turbidity up to 2.10 AU · cm-1@660 nm did not cause any interference on the 12 assays. Method comparison studies yielded slopes within 0.874–1.052 and r>0.990.
Conclusion: The new Quantia immunoturbidimetric reagents on Abbott AEROSET® and ARCHITECT c8000® system are demonstrated to be fast, reliable and accurate.

TP6.14

HYPERCALCAEMIA AN IATROGENIC CONDITION IN HOSPITAL PRACTICE

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Aim: To determine the prevalence and causes of hypercalcaemia and to assess the pattern of bone profile among hypercalcaemic subjects in a teaching hospital.
Results: Between April 2003 and April 2004, 118286 calcium requests were received from 39360 patients, an average of 3 requests/patient/year. Of these requests, the proportion with hypercalcaemia (adjusted calcium >2.60 mmol/L) was 10% (11702). Of this group, 34% had chronic renal failure (CRF), 12.5% renal transplant (RTX), 6.8% no diagnosis, 5.5% osteoporosis, 2.8% malignancy, and 1.5% primary hyperparathyroidism (HPT). The proportions of requests with Ca>3.5 and between 3.0 and 3.5 mmol/L were 0.5% and 4%, respectively. 90% were due to RF and RTX.
A significantly higher mean chloride concentration, chloride:phosphate ratio and lower anion-gap were seen in subjects with HPT and RTX compared to the other groups. Negative correlations between chloride and phosphate ($r=-0.34$, $P<0.0001$), chloride and anion-gap ($r=-0.50$, $P<0.0001$), and a positive correlation between PO4 and anion gap ($r=0.51$, $P<0.0001$) were detected.
Parathyroid hormone was significantly higher in CRF and RTX compared to the other groups (30 ± 38 and 20 ± 16 vs. 10 ± 6 pmol/L, $P<0.0001$). CTX was highest in Paget's disease (0.73 ± 0.95 vs. 0.37 ± 0.32 ng/ml, $P<0.0001$) followed by 1° HPT (0.48 ± 0.33 vs. 0.32 ± 0.34 ng/ml, $P<0.0001$) compared to other groups.
Conclusion: In this teaching hospital the commonest causes of hypercalcaemia were iatrogenic followed by malignancy and 1o HPT. Anion-gap and chloride reflect the physiological role of PTH, and highlight their potential role in the interpretation as well as the prediction of the underlying cause of hypercalcaemia.

TP6.15

SERA AND SYNOVIAL FLUID RHEUMATOID FACTORS ISOTYPES IN RHEUMATOID ARTHRITIS

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Previous findings have shown that if three rheumatoid factor (RF) isotypes are assayed instead of only IgM RF, seropositivity exceeds 90%. RF isotypes in synovial fluid (SF) were tested as additional markers for rheumatoid arthritis (RA) activity. In a cross sectional study, 59 samples of serum (S) and SF were assayed for RF isotypes. Patients were divided into two groups: 35 with moderate (MA) and 24 with severe activity (SA) and compared to 15 patients with meniscus lesions as the control group (CG). RF isotypes were measured by immunonephelometry method and monoclonal ELISA. Concentrations of RF isotypes in S and SF in CG were within the "cut off" values. Mean ± S.D. values for serum IgMRF, IgGRF and IgARF in SA were 388 ± 425, 109 ± 119, 82 ± 78 in contrast to SF: 271 ± 333, 88 ± 113, 82 ± 118, respectively. Results in serum for MA group were 74 ± 98, 63 ± 107, 35 ± 33 and in SF 41 ± 61, 32 ± 22, 37 ± 46. Mann–Whitney U test for the values of RF in S and SF, among all tested groups were statistically significant ($p<0.01$ or $p<0.05$). Values of RF were higher in SF than in serum in 25% of the MA and 66% of the SA group, which implies the possibility of intraarticular RF synthesis. The finding that 10% of IgG RF and 5% IgA RF were only elevated in SF could support the hypothesis that RF are firstly synthesized inside synovial B activated cells and can be detected several months later in the blood circulation.

TP6.16

KNOWLEDGE TESTING IN A POSTGRADUATE EDUCATION PROGRAM IN CZECH CLINICAL CHEMISTRY

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Aim: To describe the system of knowledge testing in postgraduate training in Czech clinical chemistry.
Methods: Application of European syllabus EC4 to postgraduate education of physicians and scientists. At the beginning of postgraduate education the participants pass a written test to monitor their knowledge. Each test contains 150 questions of four kinds: analytical, medical, biochemical and statistical.
Results: Postgraduate education in our country is provided for two subgroups of participants: scientists and physicians. In our example we present test No.7 for a group of 28 scientists and 18 physicians. This test contained 47 analytical, 57 biochemical, 33 medical and 13 statistical questions. Testing has been performed on a personal computer. Success in individual groups of questions and participant subgroups was as follows: Analytical questions – scientists 54.1%, physicians 46.5%. Medical questions – scientists 45.6%, physicians 67%. Biochemical questions – scientists 50.2%, physicians 61.8%. Statistical questions – scientists 39.8%, physicians 44%.
We are now preparing a new scheme for using these tests. The first test will be performed at the beginning and the second at the end of the postgraduate course.
Conclusion: The level of basic knowledge obtained in pregraduate education is not sufficient. The level of pregraduate studies seems to be better in medical schools than in the others. Physicians show a higher success level than scientists except on analytical questions.

TP6.17

SEVERE HYPERKALAEMIA (>8.0 mmol/L) IN THE NON-DIALYSIS DEPENDENT PATIENT

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Hyperkalaemia is a frequent, potentially life threatening electrolyte disorder characterised by paraesthesia, muscle weakness and natriuresis and in severe cases cardiac arrhythmias and death.
A retrospective audit of hyperkalaemia >8 mmol/L in non-dialysis dependent patients was carried out between May 1999–May 2004.
Results: 49 patients, median age 76 years (29–99 years), were identified from the data base in Clinical Biochemistry. Pseudohyperkalaemia was excluded. Significant past medical history included chronic renal failure, obstructive uropathy, congestive cardiac failure and diabetes mellitus.
Median plasma potassium concentration was 8.4 mmol/L (range 8.0–10.1 mmol/L); creatinine, 365 μmol/L (123–2418 μmol/L); urea 38.2 mmol/L (10.7–112 mmol/L); bicarbonate 14 mmol/L (3–26 mmol/L).
28 patients were on drugs known to cause hyperkalaemia. 15 were taking a single agent potentially causing hyperkalaemia and 13 were taking combinations of at least two drugs. Digoxin was also taken by seven patients, two of whom had evidence of Digoxin toxicity. Potassium-sparing diuretics were the most frequent contributory factor: Amiloride (11 cases) was taken more frequently than Spironolactone (six cases). 11 patients were receiving ACE-inhibitors or AII blockers but in only two cases were these combined with Spironolactone. 11 patients were taking either COX II inhibitors or NSAIDs.
Sepsis was the most frequent precipitant of the patients' admission or of the deterioration of their clinical condition whilst in hospital. The outcome was poor: 30/49 patients died either during the acute episode of hyperkalaemia or subsequently during their hospital admission.
Conclusions: the commonest cause of severe hyperkalaemia is a combination of deteriorating renal function, potassium-sparing drugs and sepsis. COX II inhibitors and NSAIDs are a major, under-appreciated factor.

TP6.18

EXPRESSION OF CD62L RECEPTOR ON T AND B LYMPHOCYTES IN PATIENTS WITH HYPERTROPHIED ADENOIDS IN OTITIS MEDIA WITH EFFUSION

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Adenoid hypertrophy is a common feature of childhood. The adenoid is responsible for regional immune effector and inductor in the pharynx. Its dysfunction can be a cause of otitis media with effusion.

Adhesion molecules (e.g. CD62L) are responsible for migration of lymphocytes to the pharyngeal tonsil and their activation in inflamed tissue. Most peripheral blood B and T cells express CD62L.

In this study we examined the expression of CD62L receptor in CD4, CD8 and CD19 lymphocytes in adenoid tissue in children with secretory otitis media. We examined adenoid tissue from 30 children divided into two clinical groups. The first group presented with hypertrophic adenoids and otitis media with effusion (SOM). The control group was 15 children with hypertrophied adenoid (HYP). We examined the percentage subpopulations of lymphocytes by flow cytometry method (EPICS XL, Coulter).

The percentage of CD19+CD62L+ lymphocytes was significantly higher in SOM ($59.33 \pm 9.65\%$) than HYP ($53.47 \pm 9.67\%$), $p < 0.03$. Percentage of CD4+CD62l+ was similar in hypertrophic adenoid ($57.47 \pm 6.08\%$) and in otitis media with effusion ($62.91 \pm 6.91\%$). We found no significant difference between the percentage of CD8+CD62l+ receptor in the examined group ($68.14 \pm 6.50\%$) and in the comparative group ($71.18 \pm 5.35\%$).

Because CD62L mediates lymphocyte homing to high endothelial venules of pharyngeal tonsil tissue and leukocyte rolling on activated endothelium at inflammatory sites, our results suggest that B lymphocytes with expression of CD62L receptor participates in immune response for otitis media with effusion.

TP6.19

SERUM AMYLOID A AND CRP AS A MARKER OF INFLAMMATION AND DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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Serum amyloid A (SAA) and CRP are involved in maintenance of inflammatory processes in rheumatoid arthritis (RA). To determine the comparative usefulness of SAA and CRP in the assessment of RA activity, 59 samples of serum and synovial fluid (SF) were assayed for the level of these parameters. Patients were divided into two groups: 35 with moderate (MA) and 24 with severe activity (SA), according to the established ARA criteria, and compared to 15 patients, mostly with meniscus lesion, from the control group (CG). Both parameters were measured by latex enhanced immunonephelometry method (DADE Behring). Mean values for SAA and CRP (mg/l) were 183, 53 (serum) and 42, 26 (SF) for the SA group, while in the MA group values for serum were 57 and 20 and for SF, 13 and 7, respectively, compared to 4.3 and 4.9 (serum) and 1.9 and 6.0 (SF) in CG, respectively. The Mann–Whitney U test between MA and CG in sera showed highly significant differences SAA ($p < 0.001$) in both fluids and in serum alone for CRP ($p < 0.05$). Unlike SAA, CRP is an indicator of inflammation caused by bacterial, but not viral infection which in RA could also exist especially as a consequence of immunosuppressive therapy. Monitoring the SF concentration of amyloid A is important because its secretion inside synovial tissue could induce metalloproteinase activity and additional cartilage destructions, with or without secondary AA amyloidosis.

TP6.20

EXPRESSION OF CD28 RECEPTOR ON T AND B LYMPHOCYTES IN PATIENTS WITH HYPERTROPHIC ADENOIDS IN OTITIS MEDIA WITH EFFUSION

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The hypertrophied adenoid can cause otitis media with effusion. Adhesion molecules are responsible for migration of lymphocytes to the pharyngeal tonsil and their activation in inflammatory state tissue.

CD28 is an adhesion molecule, which mediates activation of B lymphocytes. The molecules CD80 and CD86 are ligands for CD28. The binding of CD28 with ligands and TCR receptor at the same time consolidates the activation signal and enhanced secretion of IL-2 and stimulates strong proliferation of T cells.

In this study we rated expression of CD28 receptor in CD4, CD8 and CD19 lymphocytes in adenoid tissue in children with secretory otitis media.

We examined adenoid tissue from 30 children. The children divided into two clinical groups. The first group presented with hypertrophic adenoids and otitis media with effusion (SOM). The comparative group was 15 children with hypertrophic adenoid (HYP). We examined the percentage subpopulations of lymphocytes by flow cytometry (EPICS XL, Coulter).

The percentage of CD4+CD28+ lymphocytes was statistically significantly higher in SOM ($93.87 \pm 4.44\%$) than HYP ($91.01 \pm 4.63\%$), $p < 0.02$. The percentage of CD8+CD28+ was similar in hypertrophied adenoid ($66.47 \pm 8.10\%$) and in otitis media with effusion ($64.70 \pm 7.27\%$). There was no significant difference between the percentage of CD19+CD28+ receptor in the study group ($9.44 \pm 2.34\%$) and in the comparative group ($11.34 \pm 4.20\%$).

The increased percentage of helper lymphocytes (CD4+) with expression of CD28+ in the otitis media with effusion group could indicate their activation in adenoid tissue and possible migration of lymphocytes to secretory liquid in the middle ear.

TP6.21

A HYDROCARBON-ANCHORED PEPTIDE THAT FORMS A CHLORIDE-SELECTIVE CHANNEL IN LIPOSOMES

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The transport of ions through phospholipid bilayers is mediated by a variety of channels and they have a number of important physiological and cellular roles. Artificial models have been developed in an attempt to understand and mimic their behaviour, since genetic defects in channels and abnormal regulation of gating can cause a variety of human diseases, e.g. cystic fibrosis. The identification of compounds capable of transporting chloride across cell membranes has important implications in drug development.
In order to achieve and understand anion transport, naturally occurring chloride transporters were studied. Members of the ClC family of chloride protein channels contain the conserved motif GKxGPxxH in the putative anion pathway, and proline is thought to play an important role in that pathway.
A membrane-anchored heptapeptide, $(C^{18}H^{37})^2NHCOCH^2O\text{-}CH^2CO\text{-}NH\text{-}GGGPGGG\text{-}OCH^2PH$, was designed, and it has proved to be a selective chloride anion transporter that functions in phospholipid bilayer membranes. When Pro was replaced by the natural amino acid Leu, the activity decreased dramatically, releasing only about 20% the amount of Cl^- during a corresponding time period. Other peptide derivatives based on the same structural design have also been studied. Several methods were used to assay ion release including: direct measurement of chloride in the external solution surrounding phospholipid vesicles, release of the fluorescent dye carboxyfluorescein from vesicles, planar bilayer conductance, and Ussing chamber experiments.

TP6.22

SERUM LEVEL OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) IN IRON DEFICIENCY ANAEMIA

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In iron deficiency anaemia total transferrin concentration increase above the upper reference limit. Normally, transferrin contains two chains of carbohydrates that differ in the number of sialic acid residues terminally positioned. This study has investigated the serum levels of carbohydrate-deficient isoforms of transferrin (CDT) and sialic acid (SA) during iron deficiency anaemia (IDA).
Blood samples were collected from 60 women with IDA and from 20 healthy controls. CDT was estimated by anion-exchange chromatography on minicolumns followed by photometric detection of transferrin and was expressed as a percentage of total transferrin (%CDT). Sialic acid was measured by an enzymatic method. The iron status of the patients was assessed by analyses of hemoglobin, hematocrit, mean corpuscular volume and count of erythrocyte, serum ferritin, iron, total iron-binding capacity and soluble transferrin receptor.
There was no difference in the mean level of %CDT between patients with iron deficiency anaemia ($2.26 \pm 0.74\%$) and control patients ($2.05 \pm 0.37\%$). Sialic acid increased significantly from control level 0.61 ± 0.09 g/l to 0.69 ± 0.15 g/l in anaemic patients. All tested iron status markers significantly differentiated between anaemic patients with iron deficiency anaemia and control subjects. The serum sialic acid correlated positively with ferritin and soluble transferrin receptor and negatively with hemoglobin, mean corpuscular volume of erythrocyte and transferrin saturation. There was no correlation between %CDT and all markers of iron status.
The relative value of CDT (%CDT) during iron deficiency anaemia did not change because these isoforms elevate parallel with the total transferrin concentration. The increase of total sialic acid level in the sera of anaemic patients is due to the increase of transferrin, which is the sialoglycoprotein affected by the anaemia.

TP6.23

SENSITIVITY AND SPECIFICITY OF THE MARKERS OF ALCOHOL ABUSE REGARDING TO THE TIME OF ABSTINENCE

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The aim of the study was to evaluate the diagnostic sensitivity and specificity of carbohydrate-deficient transferrin (CDT), sialic acid (SA) and other biochemical and hematological indicators of chronic alcohol abuse during self-reported abstinence.
65 patients were admitted for the treatment of alcohol dependence. The blood samples were taken on admission to the hospital and after 3 weeks of hospitalization. CDT (as % CDT) was estimated using an immunoturbidimetric assay after anion-exchange chromatography and SA by enzymatic method.
Mean values of all tested markers were significantly higher on admission to hospital but only aspartate transaminase (AST), alanine transaminase (ALT) and SA returned after 3 weeks to the references limits. The diagnostic sensitivity decreased with the self-reported time of abstinence being the highest for CDT (100%), AST (70%), ALT (50%) and gamma-glutamyl transferase (GGT) (50%), when alcohol was consumed in the last week. For sialic acid these values were highest after 2 weeks of abstinence and for mean corpuscular volume (MCV) after 1 week. The specificity of above tests did not change during abstinence and was the highest for MCV (100%), followed by sialic acid, GGT and ALT (96.7% each), CDT and AST (93.3% each).
We can state that the diagnostic sensitivity for tested laboratory markers except sialic acid depends on the self-reported time of abstinence, diminishes in parallel with the prolongation time of alcohol withdrawal. Of all tests, CDT has the highest and sialic acid the lowest sensitivity when alcohol was consumed in the last week. The specificity of the above tests did not depend on the time of abstinence.

TP6.24

RELATIONSHIP BETWEEN SALIVARY AND PLASMA MALONDIALDEHYDE LEVELS AND PERIODONTAL STATUS IN HEALTHY SUBJECTS AND PATIENTS WITH PERIODONTAL DISEASE

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Periodontal disease is becoming more common nowadays, but molecular mechanisms of its pathogenesis are still lacking. Recent experimental studies have suggested involvement of lipid peroxidation and oxidative cell damages. In this study we analyzed both salivary and plasma malondialdehyde (MDA) levels, as indicators of local and systemic disorders and examined their relations to the indicators of the periodontal status (DP [dental plaque index], GI [gingival index], RI [Ramfjord index] and TM [tooth mobility index] in humans. Patients with progressive periodontal diseased (P-group, $n=15$) and healthy individuals (C-group, $n=7$) were investigated. Indicators of periodontal status were determined by standard procedure, while MDA was analyzed by colorimetric method with thiobarbituric acid. The results indicate that in parallel with development of the periodontal disease indicated by significant ($p<0.05$) increase of DP (C-group 0.04 ± 0.01 scores, P-group 1.37 ± 0.63), GI (C-group 0.06 ± 0.02 scores, P-group 1.65 ± 0.52), RI (C-group 0.31 ± 0.34 scores, P-group 4.05 ± 0.65), and TM (C-group 0.24 ± 0.04, P-group 1.60 ± 0.84), there was a significant increase of both plasma (from 2.70 ± 1.27 μmol/l in controls to 7.43 ± 3.02 μmol/l in P-group), and salivary MDA levels (from 2.03 ± 0.87 μmol/l in controls to 5.56 ± 3.85 μmol/l in P-group). In addition salivary MDA levels correlated ($p<0.05$) with DP ($r=0.444$), GI ($r=0.458$) and RI ($r=0.413$), but not ($p>0.05$) with TM ($r=0.060$). These results suggest that lipid peroxidation could be the basic lesion leading to further tissue damage and destruction of the periodontal tissues that finally result in loss of teeth. In conclusion, oxidative stress seems to be an important molecular mechanism involved in development of periodontal disease that should be considered in therapy.

TP6.25

ULTRASENSITIVE C-REACTIVE PROTEIN IN ASCITES AS INDICATOR OF ANTIBIOTIC TREATMENT IN PATIENTS WITH SPONTANEOUS BACTERIAL PERITONITIS

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Spontaneous bacterial peritonitis (SBP) is a frequent complication in patients with cirrhosis and ascites, with a life expectancy 5–6 times lower than non-infected cirrhotic patients. They have a high mortality (30 to 50%), with 20 to 50% recurrence probability during the first year. Ultrasensitive CRP (CRPu) is an excellent tracer of the inflammatory process and rises quickly.
Antibiotic treatment response through measurement of CRPu level in peritoneal fluid was evaluated.
Ascitic fluid (AF) in patients with cirrhosis and ascites was examined as follows: CRPu by inmunoturbidimetry, Lactate Dehydrogenase (LDH) in AF and blood, AF leukocyte count in Fuchs-Rosenthal chamber, and AF culture. SBP was defined as AF with polymorphonuclear (PMN) count $>250/mm^3$ and LDH blood serum/AF >1 in absence of other intra-abdominal infectious disease. Two groups were established: A (PMN count $>250/mm^3$, [$n=109$]) and B (same patients as group A, 48 h of antibiotic treatment). A group was subdivided into A1 (negative culture [$n=81$]), and A2 (positive culture [$n=28$]). B also was subdivided into B1 (PMN count $<250/mm^3$, [$n=87$]), and B2 (PMN count $>250/mm^3$ [$n=22$]).
Mean concentrations of CRPu were (mg/L): Group A1 21.22 ± 6.97; Group A2 23.07 ± 6.20; Group B1 2.21 ± 0.55, Group B2 28.13 ± 9.68. No statistically significant differences ($p>0.05$) were found between groups A1-A2; statistically significant differences ($p<0.001$) were found between groups A1-B1, A2-B1 and B1-B2. CRPu in AF of patients with SBP does not discriminate between cultured positive and negative AF ($p>0.05$). Nevertheless, it proved to be an excellent indicator of antibiotic efficacy in patients with SBP ($p<0.001$).

TP6.26

MECHANISM STUDIES ON GLYCERYL TRINITRATE INHIBITS HELA CELLS PROLIFERATION

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Organic nitrates have been used for the treatment of cardiovascular diseases for more than 100 years and these drugs are still amongst the most frequently prescribed and applied drugs worldwide. Development of tolerance against the hemodynamic effects of nitrates during sustained therapy, however, limits their clinical application. Nitrate tolerance has been shown to increase superoxide and peroxynitrite production that might lead to protein and lipid oxidation in tissues and cell. We investigated the effect of glyceryl trinitrate (GTN) on proliferation of tumor cells and its mechanism. The Human Cervical Carcinoma Cell Line (Hela) was used as experimental model. MTT assay was used to evaluate the proliferation activity of Hela cells. Bcl-2 protein, cell cycle and apoptosis index in cultured Hela cells were analyzed by flow cytometer. Nitrite and reduced glutathione (GSH) were measured with Griess and DTNB colorimetric method. The results showed that GTN metabolized to nitric oxide (NO) and nitrite and initiated the depression of proliferation activity in cultured Hela cells. When the cultured Hela cells were treated with 0.175 mmol/L GTN the GSH in cells decreased from $0.164+/-0.003$ to $0.136+/-0.003$ mg/dl and the GSH in culture medium decreased from $2.21+/-0.093$ to $1.80+/-0.025$ mg/dl. In addition, bcl-2 protein decreased and apoptosis index increased (from $1.47+/-0.03$ to $2.72+/-0.089$), cell cycle arrest in G2-M phase was found in Hela cells treated with GTN. We conclude that GTN possesses inhibitory effects on the proliferation of Hela cells and the mechanism is related to depletion of GSH and release of nitric oxide.

TP6.27

PELGER–HUET ANOMALY: A HAEMATOLOGICAL PHENOMENON OR AN ANOMALY CONNECTED WTH GENETIC MALFORMATIONS?

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Pelger–Huet anomaly is an autosomal dominant hereditary disorder. It is caused by mutation of lamin B receptor (LBR) located on chromosome 1. This receptor (by helping cholesterol synthesis) becomes a structural base of the cell's membrane. Some syndromes caused by impairment of sterol biosynthesis have been reported. Among these, Greenberg dysplasia has LBR receptor mutations connected with Pelger anomaly. Although, in case of heterozygous individuals, the Pelger anomaly seems a harmless phenomenon, examination of animal and human homozygotes suggests that other genetic malformations and death may be associated with it. This reinforces the probability that modified forms of LBR, or its decreased expression, may cause an altered membrane function and embryonic developmental problems.
In our hospital we found a Pelger phenomenon in the full blood count of a child with Fryns syndrome. This is a new contribution to establish a possible aetiology of malformations with unclarified genetic background. Is Fryns syndrome related in some way to Greenberg dysplasia or even perhaps the same genetic mutation with different symptoms?

TP6.28

MATERNAL SERUM SCREENING FOR DOWN SYNDROME IN THAI PREGNANT WOMEN

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Down syndrome is one of the most important causes of mental retardation in the population. The main aim of this pilot study was to evaluate the sensitivity of maternal serum screening (triple test) to identify women at an increased risk for an affected pregnancy and to reduce the incidence of invasive amniocentesis procedures. Triple test involves combining the maternal age risk with the risks associated with the concentrations of maternal serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin that are measured by a chemiluminescence immunoassay method.
The study consisted of 1000 pregnant women, attending antenatal care unit, Hat Yai Center Hospital. The gestational range for the study group was 14–19 weeks. 171 of these women were considered at increased risk for Down syndrome and 141 of them had an amniocentesis. Among 199 pregnant women over 35 years of age, 93 of them were triple test positive. The results of karyotyping show that there were 4 cases with trisomy 21, 1 case with 47 XXX and 1 case with chromosome translocation. All these chromosome abnormalities were detected in pregnant women over 35 years of age.
In conclusions, the use of triple test as a screening tool in our population could reduce the number of amniocenteses, while no cases of Down syndrome would be missed.

TP6.29

RAMAN SPECTROSCOPY AS A SCREENING METHOD FOR DRUGS OF ABUSE AND THEIR PRECURSORS

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Identification of illicit drugs, namely MDA (3,4-methylenedioxyamphetamine) and MDMA (3,4-methylenedioxymethamphetamine), is usually carried out using techniques such as colorimetric tests, GC-MS or NMR spectroscopy. However, these methods have proved to be poorly selective, expensive and often time-consuming when compared to vibrational spectroscopy (e.g. Raman), which provides unique fingerprint spectra for each different compound analysed.
The present work reports a Raman spectroscopic study, coupled to theoretical (ab initio) calculations, of several β-methyl-β-nitrostyrene derivatives, which are important intermediates in the synthesis of illicit amphetamine-like drugs.
The Raman spectra obtained showed characteristic features for each of the compounds studied. Moreover, it was verified that their vibrational pattern is strongly affected by the presence of a para substituent in the aromatic ring (either O-CH3 or S-CH3), as well as by an O $\rightarrow$ S substitution. Based on the complete ab initio conformational analysis performed for these systems, a thorough assignment of the experimental spectroscopic data was performed, leading to a ready and unequivocal differentiation and identification of this kind of synthetic precursors of illegally produced drugs of abuse – namely through the bands at 1300 cm^{-1} (NO2 stretching modes), as well as at 250 and 1440 cm^{-1} (typical of the CH3 group).
The described results indicate that Raman spectroscopy is a most promising tool for Forensic Sciences, as a screening method for determining the composition profiles of illicit substances, as well as for tracking clandestine laboratories. Thus, it will hopefully be possible, in the near future, to rely on a Raman database that will constitute an invaluable tool for both forensic control and toxicological studies.

TP6.30

NEW INSIGHTS ON COCAINE–OPIATES INTERACTIONS

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The simultaneous self-administration of opiates and cocaine–"speedballing"–is relatively widespread among drug users, and is probably responsible for higher levels of euphoria compared to those produced by each drug separately. Although the underlying biological basis for abuse of cocaine and opiate combinations is still unclear, clinical studies can give some insight into the desire for this dual abuse.
Up to this date, the pharmacological reasons for cocaine use in opiate-dependent individuals are poorly understood, and little is known about the patterns of cocaine and heroin or morphine co-use. Thus, the present study was undertaken, using electrochemistry, Raman and NMR spectroscopy, in order to investigate the possible interactions between opiates and cocaine, at a molecular level.
The results obtained by any of the methods used reflect a chemical interaction between cocaine and morphine, but not between cocaine and heroin. This specific cocaine:morphine interaction was detected

in solution through electrochemical methods, and confirmed by both RMN and Raman spectroscopy coupled to theoretical methods. In fact, the structural data yielded by the ab initio calculations allowed the conclusion that the presence of the two terminal −O(C=O)CH3 groups in heroin seem to hamper the approximation of the cocaine molecule, while the approach to the morphine cavity was found to be much more favourable, as it does not involve any significant steric hindrance. Furthermore, only the protonated species of morphine, for which a slightly more open conformation was determined, leads to a detectable association with cocaine. The cocaine–morphine interplay is thus suggested to take place through the inner cavity of the morphine molecule, most probably through a (C=O)OH . . . O interaction.

TP6.31

CLUSTERIN IS DIFFERENTIALLY EXPRESSED IN MALIGNANT MELANOMAS AS COMPARED TO ACQUIRED MELANOCYTIC NEVI AND MODULATES UV-B-INDUCED APOPTOSIS IN VITRO

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Aim: To establish a connection between clusterin (CLU) expression and malignant melanoma, and to see how this expression is regulated.
Methods: Paraffin sections of primary cutaneous malignant melanomas, metastases of malignant melanomas and acquired melanocytic nevi were analyzed immunohistochemically using antibodies that detect both isoforms of clusterin. The pro-apoptotic nCLU and the anti-apoptotic sCLU were detected in a proportion of malignant melanomas and metastases, but not in acquired melanocytic nevi. Additionally, expression of CLU in various melanoma cell lines (MeWo, SKMEl-28, SKMEL-5, SKMEL-25, and MelJuSo) was also analyzed.
Results: All melanoma cell lines revealed strong expression of CLU mRNA and protein. CLU mRNA and protein levels were regulated time-dependently by 1,25(OH)2 D3 treatment. Moreover, stable transfected and CLU over-expressing LNCaP CLU+ prostate carcinoma cells responded differentially as compared to untransfected LNCaP cells, analyzing cell cycle and apoptosis following UV- or 1,25(OH)2 D3-treatment. In contrast to benign acquired melanocytic nevi, CLU is expressed in malignant melanomas, metastases and melanoma cell lines. CLU expression is regulated time-dependently by 1,25(OH)2D3, indicating that antiproliferative effects of 1,25(OH)2D3 on melanoma cell lines may be at least in part mediated via regulation of CLU expression.
Conclusion: CLU may be of importance for the growth characteristics of melanoma cells, and CLU over expression protects against the cytotoxic effects mediated by 1,25(OH)2 D3 in prostate LNCaP cell lines. CLU seems to be important for the treatment of melanoma. However, its role must be further defined by more experiments.

TP6.32

BRITISH THORACIC SOCIETY GUIDELINES FOR BIOCHEMICAL ANALYSIS OF PLEURAL FLUID–A DISTRICT GENERAL HOSPITAL PERSPECTIVE

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In 2003 the British Thoracic Society published guidelines for the investigation of unilateral pleural effusions, aiming to establish a swift diagnosis while minimising unnecessary invasive investigations.
An algorithm was developed at Southend Hospital; the samples required on all patients were pleural fluid for protein and LDH, a blood gas syringe sample for pH and a paired serum sample for LDH and protein. Glucose measurement should only be requested on rheumatoid or connective tissue effusions and required paired pleural fluid and plasma glucose fluoride samples.
An audit covering six months was carried out to measure adherence to the sample requirements; assess whether Light's criteria discriminated between transudates and exudates better than pleural fluid protein alone; assess if pH measurement was clinically useful on all fluids.
Only 70% of requests had a paired serum sample and only 21% were accompanied by a suitable sample for pH. 42% requested glucose but only 18% had the correct samples collected.
The patients' case notes were reviewed to ascertain the clinical diagnosis. Clinically 71% of effusions were exudates and 25% transudates. Using pleural fluid protein alone the sensitivity for exudates was 51% which improved to 83% using Light's criteria.
Only 21% of patients had pH measured and in 18% (4 patients) indicated the need for aggressive treatment, two due to complicated parapneumonic effusions.
The samples recommended in the guidelines were not received in all patients; insufficient numbers of patients had pH measured, so conclusions cannot be drawn on the usefulness of pH in all patients. Excessive numbers of glucose requests were received.

TP6.33

SW AND WESSEX REGIONAL SURVEY OF CARDIAC MARKERS

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The definition of myocardial infarction has been evolving over recent years. The new definition includes elevated troponin levels. Currently there are no published UK guidelines regarding the frequency and time intervals for troponin sampling. The choice of myocardial infarction (MI) and risk based thresholds for troponin measurements are still debatable. The aim of this survey was to review current practice in the SW and Wessex region laboratories.
In September 2004 a questionnaire was sent out to 21 laboratories, and 20 responses were received. All laboratories offered troponin, with 80% offering 'on demand' service, and 55% having a turn

around time of 1 h or less. Most laboratories that do not provide 'on demand' service for troponin have point of care testing available in the hospital, which in 80% of sites is supported by the lab for EQA. 10% of labs routinely offer two troponin measurements per patient episode. There is a wide variation in quoted reference ranges and threshold values for supporting a diagnosis of acute coronary syndrome (ACS) and MI for the major troponin methods. Therefore classification of some patients presenting with chest pain or ruling out cardiac damage is hospital dependent.

25% of hospitals do not have a documented chest pain protocol. 45% of laboratories had been involved in auditing their service, although there was minimal evidence provided that this has led to change in practice. Financial restrictions offer significant impediments to improving service provision in 30% of hospitals.

This survey highlights the variable provision of troponin services within the SW and Wessex region, and the need for evidence-based UK standards.

TP6.34

RELATION BETWEEN METABOLIC CELLULAR DAMAGE AND SEVERITY OF BLAST TRAUMA

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The osmolality "gap" (OG) is a valuable indicator of metabolic cellular damage in trauma. We evaluated the relationship between this parameter and the severity of trauma in bombing casualties. Fifty-two patients injured in a 1999 bombing with Injury Severity Score (ISS) from 3 to 66 were divided into two groups: group A with ISS$<$25 (n=24) and group B with ISS$>$25 (n=28). The control group consisted of 10 uninjured volunteers. Venous blood was sampled daily for 10 days after injury. Osmolality (OSM) was measured using an osmometer (Knauer) and OPP calculated from OPP=1.86 $\times$ sodium (mmol/L)+9+glucose (mmol/L)+urea (mmol/L). Serum water content varies with total protein and lipids concentration in critically ill patients. OSM and OPP were corrected using factor R ($R=C/F$, C=water content in control group, F=water content in the serum of injureds). Serum water content F (kg/L) was calculated using Waugh's equation: $F=\{991-1.03\times\text{total lipids (g/L)}-0.73\times\text{total protein (g/L)}\}/1000$. OG was significantly higher in both group A (11.0 ± 8.8 mosm/kg, $p<0.05$) and group B (13.8 ± 12.0 mosm/kg, $p<0.01$) than in the control group (0.99 ± 2.4 mosm/kg). OSMR in groups A and B did not differ from that in control group, while OPPR was lower in both groups ($p<0.05$). However, there was no significant difference between groups A and B in the levels of OSMR, OPPR and OG. No correlation was observed between these parameters and ISS. The most significant observation of this study was the multiple increase in OG of the injured, regardless the severity of injury. Similar OG values in both groups indicated severe metabolic cell damages, which could both disturb final recovery and cause permanent damages of some tissues.

TP6.35

NITROTYROSINE SPUTUM GRANULOCYTE AND ALVEOLAR MACROPHAGE IMMUNOPOSITIVITY IN PATIENTS WITH ACUTE LUNG INFECTION AND LUNG CANCER

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Objective: Nitrotyrosine (NT) is a marker of inflammation and nitric oxide (NO) production and damage. Analyzing differences in inflammatory cells and their cytotoxic effects between infections and cancer may contribute to better understanding of cancer development. The aim was to analyze NT positive (NT+) sputum granulocytes and alveolar macrophages (AMS) in patients with acute lung infection and in patients with lung cancer.

Patients and methods: Immunocytochemical microscopic analysis of NT+ sputum granulocytes and AMS was done in 19 patients with acute bacterial lung infection (ABLI) and in 31 patients with lung carcinoma.

Results: Patients with ABLI had significantly higher percentages (mean 36, range 0–92) and scores (38, 0–161) of NT+ granulocytes in comparison to the same parameters in patients with carcinoma (percentages: 12, 0–66; scores: 14, 0–76). However, there were no significant differences between percentages and scores of NT+ AMS between both groups of patients. A marginally significant difference was only found for percentages and scores of NT+ AMS between a subgroup of patients with cancer before (percentages: 13, 0–60; scores: 14, 0–77) and after (percentages: 3, 0–32; scores: 3, 0–32) cytostatic therapy.

Conclusion: Results indicate that NT was highly expressed in sputum granulocytes in patients with ABLI in comparison to patients with lung cancer. However, similar NT+ AMS in both groups of patients could probably be connected to the involvement of AMS and NO-dependent damage. Further studies will clarify if reduced NT+ AMS in patients with cancer after cytostatic therapy is connected to impaired immunoreactivity or to good therapy response.